The Effect of Soaking Almonds and Hazelnuts on Phytate and Mineral Concentrations

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Abstract

Background: Nuts are important sources of macronutrients, in particular cis-unsaturated fats; micronutrients; and phytonutrients, which are all important components of a cardioprotective diet. However, one phytonutrient, phytate, has been associated with reduced bioavailability of some minerals, including zinc, iron, calcium and magnesium. Recently, the general public has been bombarded with messages advocating soaking (or "activating") nuts in order to enhance their health benefits. However, there is currently no scientific evidence to support or refute such claims. Research on grains and legumes has shown reductions in phytate concentrations with soaking, particularly when particle size is reduced. Therefore, the overall aim of this study is to assess the effects of different soaking regimes on phytate and mineral concentrations of whole and chopped almonds and hazelnuts to inform messages around soaking nuts.

Methods: Two nut types, almonds and hazelnuts were analysed in this study in two different forms (whole and chopped). Three different soaking treatments were used to assess the importance of soaking time and the addition to salt to the soaking solution: 1. soaking for 12 hours in salt solution (12hrs+salt), 2. soaking for 4 hours in salt solution (4hrs+salt), and 3. soaking for 12 hours in water with no added salt (12hrs-salt). These were compared to unsoaked whole nuts. All samples were analysed for phytate (sum of inositol hexa-phosphate (IP6) and inositol penta-phosphate (IP5)), calcium, magnesium, phosphorous, potassium, iron, sodium, and zinc. Phytate concentrations were analysed using high-performance liquid chromatography (HPLC) and minerals by inductively coupled plasma mass spectrometry (ICP-MS).

Results: No statistically significant differences in phytate concentrations were observed between any of the treatments for whole almonds and whole hazelnuts. However, for

chopped nuts, the soaking process generally resulted in statistically significant decreases in phytate concentrations, with reductions around 10% in hazelnuts (all $p<0.001$). In addition, statistically significant reductions were also observed for most minerals (calcium, iron, magnesium, phosphorus, potassium and zinc) following soaking in chopped nut. The reductions in phytate concentrations in the chopped nuts were accompanied by a reduction in mineral content which attenuated reductions in the phytate:mineral molar ratios. Hence, the changes in the phytate:mineral molar ratios suggest that soaking both whole or chopped nuts had no meaningful effect on the bioavailability of minerals. Changing the soaking duration, and addition of salt to the soaking solution, generally had little effect on mineral concentrations, with a few exceptions. However, an increase in sodium content was seen for whole (around 200-300 fold) and chopped (around 600-800 fold) in both almonds and hazelnuts when soaked in salt solutions compared to unsoaked nuts and nuts soaked without salt (all $p\leq0.002$).

Conclusion: It is evident from the current research that soaking almonds and hazelnuts in the whole form was not effective in reducing phytate concentration. While soaking chopped nuts led to reductions in phytate, the mineral content was also compromised, with no overall improvements observed in the phytate:mineral molar ratios. Therefore, there is no evidence to support claims that activating nuts reduces phytate content to the extent which allows for greater nutrient bioavailability.

Preface

This study was part of a collaborative project 'The Soaking Nuts And Phytate' (SNAP) study and is one in series of a group of studies that evaluated the effect of soaking different types of nuts (almond, hazelnuts, peanuts and walnuts) on phytate, vitamin and mineral composition and water activity.

Academic supervisors included Dr Rachel Brown, Kirsten Webster, Dr Karl Bailey, Andrew Gray and Dr Alex Chisholm. Dr Rachel Brown, Kirsten Webster, and Andrew Gray were responsible for developing the study protocol, guidance with data analysis and providing feedback throughout thesis writing. In addition, Andrew Gray performed the study sample calculation and statistical analysis. Dr Karl Bailey assisted with phytate analysis, and Dr Malcolm Reid was responsible for mineral and trace element analysis. This project was under taken in conjunction with another student, Kylie Ann Han Kelvin, who assisted with chopping, soaking, drying, weighing, grinding of nuts as well as weighing and packing samples for analysis.

The candidate had the primary responsibility for analysis of phytate and minerals in Almonds and Hazelnuts. Responsibilities include:

- Overall management of the project
- Conducting a literature review
- Preparation of nuts; including chopping, soaking, drying, weighing, grinding, weighing and packing samples for analysis
- Assisted with phytate analysis
- Data entry
- Interpretation and write-up of results
- Preparing the thesis

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List of Abbreviations

Abbreviation	Meaning
BMI	Body Mass index
BP	Blood Pressure
CAD	Coronary Artery Disease
CHO	Carbohydrates
CI	Confidence interval
CV	Coefficient Variation
CVD	Cardiovascular Disease
dw	Dry weight
g	Grams
HCl	Hydrochloric acid
HDL	High Density Lipoprotein
HPLC	high-Performance liquid chromatography
Hrs	Hours
ICP-MS	Inductively coupled plasma mass spectrometry
IHD	Ischemic Heart Disease
IP5	Inositol penta-phosphate
IP6	Inositol hexa-phosphate
kCal	Kilo calories
kJ	Kilojoules
kJ/g	Kilojoules per gram
Kpa	Kilopascals

mg	Milligram
µg	Microgram
MI	Myocardial Infarction
mL	Millilitres
Mm	Millimeters
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
Rpm	Revolutions per minute
SFA	Saturated fatty acid
SNAP	The ‘Soaking Nut And Phytate’ study
T2DM	Type 2 Diabetes Mellitus
Tbsp	Tablespoon
Tsp	Teaspoon

1 Introduction

Nuts are nutritionally rich, abundant in protein, fibre, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), as well as several vitamins (e.g. Vitamin A, Vitamin E, Vitamin B6, niacin, folate), minerals (e.g. calcium, magnesium, potassium, iron, selenium, zinc) and phytonutrients (1). Regular nut consumption has consistently been associated with lower all-cause mortality and cardiovascular disease (CVD) (2-11). Reductions in CVD observed with nut consumption are largely attributed to their cholesterol-lowering effects (11). Currently the Ministry of Health dietary guidelines recommend 30 g of nuts be consumed daily to provide maximal benefits without influencing body weight (12, 13). However, nut consumption among the New Zealand population has been found to be relatively low (e.g. the prevalence of daily whole nut consumption is 2.8 g in the population and 40.3 g among nut consumers (14). Nuts also contain bioactive compounds called phytochemicals, of which some have anti-nutrient properties. Although there are a number of anti-nutrients, phytate is most commonly found in cereals, legumes and nuts (15). Phytate affects the bioavailability of minerals such as iron, zinc, calcium and magnesium when the phytate: mineral ratio is greater (16). This is primarily of concern if the intakes of these minerals are low or if an individual has a vegetarian or vegan diet where consumption of phytate containing foods are high.

Recently, the public has been bombarded with information in the lay media advocating various methods of ‘activating’ nuts to provide maximal health benefits. The term ‘activating’ refers to neutralization of enzyme inhibitors present in nuts, hence, allowing greater nutrient bioavailability, proper digestion and changes in texture and flavour of the nut (17-21). While there are vast amounts of information in lay literature on

‘activating’ nuts, there is no scientific evidence to support or refute such claims. Also, there is no consensus on the best method for activation, although the majority of protocols suggest soaking nuts in salted water for approximately 12 hours, followed by drying for 24 hours (20, 22, 23). It is claimed that salt aids in activating enzymes that are responsible for deactivating the enzyme inhibitors in nuts (19, 20, 23). Therefore, it is essential to examine the effects of activating nuts on phytate and mineral content in order to inform evidence-based guidelines regarding the optimisation of the overall nutritional value of nuts. This is important because ‘activating’ nuts is a time-consuming process, and could inadvertently be a barrier to regular nut consumption. This is of concern given that current nut intakes are lower than recommended. Previous research in grains and legumes has reported reductions in phytate concentrations with soaking, especially when combined with particle size reduction (24-34). However, soaking was also found to increase the leaching of water-soluble vitamins and minerals in grains and legumes, especially when particle size was reduced (24, 26, 29, 30). Therefore, it is of interest to investigate the effects of soaking on both whole and chopped nuts. Furthermore, given the process of soaking nuts is time-consuming, assessing the effect of soaking for a shorter duration on phytate and mineral concentrations would also be beneficial. Lastly, given that commonly recommended soaking protocol recommend the addition of salt, it is of interest to examine whether this addition does make a difference to the phytate and mineral content of soaked nuts. Therefore, the overall aim of this study is to assess the effects of different soaking protocols, varying in length and salt content on phytate and mineral concentrations (iron, zinc, magnesium, potassium, phosphorus, calcium and sodium) in whole and chopped almonds and hazelnuts.

2 Literature Review

2.1 Effects of nuts on health: An overview

Several recent meta-analyses have shown that nut consumption is inversely associated with all-cause mortality (3, 4, 6, 7, 9, 10). While the majority of studies are conducted among well-educated and/or European populations, recent analysis of three cohorts led by Luu et al. showed an inverse association between nut consumption and total mortality across different racial/ethnic groups and low socioeconomic groups (7). Due to the observational nature of these studies, cause and effect of the inverse association between nut consumption and all-cause mortality cannot be determined. Overall, however, the literature to date consistently suggests higher nut consumption is beneficial, it is associated with a reduction in all-cause mortality in several different populations regardless of gender and socioeconomic status.

The inverse association observed for total mortality appears to be predominantly driven by reductions in cardiovascular disease (CVD) mortality. Numerous meta-analyses have consistently reported reduced CVD incidence and mortality with nut consumption (2, 4-9, 11), with a protective effect found when 2 servings of nuts were consumed weekly compared to consumption of no nuts. It appears from intervention studies that the reductions in CVD observed with nut consumption are largely related to the cholesterol-lowering effects of nuts (35). Research suggests the cholesterol lowering effects of nut consumption is dose related, and more pronounced in participants with higher baseline low density lipoprotein (LDL) cholesterol or lower body mass index (BMI) (35). Furthermore, nut consumption was also inversely associated with several cardiovascular disease mediators, such as inflammation, oxidative stress, and endothelial dysfunction (36). It should be noted that many of the prospective studies also report that nut

consumers are leaner, less likely to smoke, more likely to exercise, consume more fruits and vegetables, and are more likely to use multivitamin supplements and therefore these factors are likely to be confounding the association. Despite some studies adjusting for combination of these variables in their analyses, there may still be residual confounding from omitted or imperfectly specified variables.

There is less research on the association of regular nut consumption and other diseases. The effects of nut consumption on stroke remains unclear, with most studies reporting no effect (2, 4, 6, 7, 9, 11) while some report a reduction in the risk of stroke, especially in women (37, 38).

Epidemiological studies examining the association between nut intake and cancer risks have produced inconsistent results (39, 40). Recent meta-analyses have shown an overall statistically significant reduction in the risk of overall cancer incidence and mortality (3, 4, 10, 41). Recently, Wu et al. found a statistically significant association for some cancers (colorectal, endometrial, pancreatic) but not others (41). Collectively, studies tend to report statistically significant associations between nut consumption and reduced risk of cancer incidence and mortality, however, further research is required on the effects of nut consumption on specific types of cancer.

Research on nut consumption and type 2 diabetes mellitus has produced mixed results, with the majority reporting no associations (2, 6, 7, 11, 41-45). A pooled analysis of four studies by Luo et al. reported a 12% (95% CI: 8%, 16%) risk reduction for a 1 serving/ day increment in nut consumption, however this inverse association was substantially attenuated when adjusted for BMI (6). In contrast, a meta-analysis by Afshin et al. showed a 13% (95% CI: 6%, 19%) decrease in diabetes risk for every four additional servings of nut consumed per week (2). However, this inconsistent result

could be attributed to incomplete adjustments of variables such as BMI and other confounding factors.

Overall, there is consistent evidence for an inverse association between regular nut consumption and all-cause mortality and CVD, with more research required to gain a better understanding of the relationship with nut intake and other diseases.

2.2 Nutritional components of nuts

Nuts are defined as dry fruits containing seeds in which the ovary walls become hard at maturity (1). Commonly consumed nuts include tree nuts such as almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios and walnuts (1). In general nuts are high in fat and low in CHO, although, the proportions of the different types of fat are unique for each nut. Although, chestnuts are also classified as tree nuts; they have a nutrient composition which is very different from other tree nuts (i.e. higher in carbohydrate (CHO) and water) and hence are not included in this thesis (46, 47). This is also apparent for coconut, which has a high saturated fatty acid (SFA) content (48). Peanuts, although botanically classified as a legume, have a very similar nutrient composition to tree nuts, and so – for the purposes of this thesis, are also classified as nuts (1).

Nuts are nutrient rich, providing macronutrients such as protein, fibre, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (**Table 1**), as well as micronutrients including vitamins (Vitamin A, Vitamin E, Vitamin B6, niacin, folate), minerals (calcium, magnesium, potassium, iron, selenium, zinc) (**Table 2**) (1).

Despite having a high fat content (20-30 kJ/g), nuts contain low levels of saturated fatty acids (1, 36). The predominant fatty acids found in nuts are MUFA and PUFA (linoleic acid and alpha- linolenic acid) (1, 36). Most nut types are high in MUFA. Walnuts

Table 1: Macronutrient consumption per 100g of nuts

Macronutrients	Almonds	Brazil nuts	Cashews	Hazelnuts	Macadamias	Peanuts*	Pecans	Pine nuts	Pistachios	Walnuts
Energy (kJ) (kCal)[1]	2270 (568)	2790 (698)	2400 (600)	2550 (638)	2970 (743)	2480 (620)	2870 (718)	2500 (625)	2490 (623)	2890 (723)
Protein (g)[1]	21.2	12	17	14.8	9.8	23.7	7.7	24	20.6	25.7
CHO (g)[1]	4.6	3.8	16.8	5.2	4.5	13.9	13.8	12.6	7.7	4
Fibre (g)[1]	12.2	8	5.9	10.4	9.3	8	7.6	4.9	10.8	6.4
Total fat (g)[1]	49.4	68.2	49.2	59.8	73.7	49.7	67.6	50.7	54.4	64.5
SFA (g)[1,2]	3.7 (7.5%)	17.4 (25%)	8.4 (17%)	5.7 (9%)	11 (15%)	6.9 (14%)	5.4 (8%)	7.8 (15%)	6.9 (13%)	6.5 (10%)
MUFA (g)[1,2]	30.9 (63%)	22.4 (33%)	31.1 (63%)	42.4 (71%)	58.2 (79%)	24.6 (49%)	42.2 (62%)	19.2 (38%)	36.8 (68%)	12.4 (19%)
PUFA (g)[1,2]	12.1 (24%)	25.4 (37%)	7.5 (15%)	8.7 (14%)	1.3 (2%)	15.7 (32%)	16.7 (25%)	21.5 (42%)	8.3 (15%)	42.5 (66%)
Linoleic acid (g)[3]	12.2	20.5	7.7	7.8	1.3	15.6	20.6	33.2	13.2	38.1
Alpha- linolenic acid (g)[3]	0	0.05	0.15	0.09	0.21	0	1	0.16	0.25	9.08

1. Source: The Concise New Zealand Food Composition Table 11th Edition (48)
2. Percentage of total fat (%)
3. Source: Ros E, Health Benefits of Nut Consumption (1)

Abbreviations: CHO, carbohydrate; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; g, Grams; kJ, Kilojoules; kCal, kilo calories

* Nuts, peanuts, all types, dry roasted, no added salt

Table 2: Micronutrient composition per 100g of nuts

Micronutrients	Almonds	Brazil nuts	Cashews	Hazelnuts	Macadamias	Peanuts*	Pecans	Pine nuts	Pistachios	Walnuts
Vitamin A (µg)[1]	Trace	2	1	3	0	0	4	2	22	4
Beta-Carotene (µg)[1]	1	9	6	16	0	0	25	10	130	21
Vitamin C (mg)[1]	0	0.7	0	1	0	0	2	2	7	3.1
Vitamin E (mg)[1]	26	7.2	0.73	17	0.41	7.1	6.6	14	2.7	15
Vitamin B6 (mg)[1]	0.14	0.17	0.35	0.16	0.2	0.26	0.19	0.34	0.34	0.92
Thiamine (mg)[1]	0.21	1	0.64	0.48	0.35	0.44	0.85	0.81	0.82	0.32
Riboflavin (mg)[1]	1	0.12	0.19	0.08	0.11	0.1	0.13	0.19	0.17	0.07
Niacin (mg)[1]	7	4.3	7.3	6.7	3.8	17	1.8	8.7	5.8	5.9
Folate (µg)[1]	50	22	25	110	11	150	22	58	51	66
Potassium (mg)[1]	710	760	550	900	370	660	390	600	1100	580
Phosphorus (mg)[1]	480	590	530	280	140	360	290	510	500	320
Calcium (mg)[1]	260	180	34	180	70	54	36	26	140	130
Magnesium (mg)[2]	275	376	292	163	130	168	121	251	121	158
Iron (mg)[1]	3.7	2.8	5	2	2.4	2.3	2.1	9.2	6.8	3.3
Zinc (mg)[1]	3.1	4.2	5.5	2.1	1.7	3.3	5.5	4.3	1.4	2.3
Selenium (µg)[1]	2.5	1300	33	76	7	7.5	5.3	5.3	5.3	58

1. Source: The Concise New Zealand Food Composition Table 11th Edition (48)
2. Source: Ros E, Health Benefits of Nut Consumption (1)

Abbreviations: mg, milligram; µg, microgram.

* Nuts, peanuts, all types, dry roasted, no added salt

provide the richest source of alpha- linolenic acid whereas pine nuts provide rich source of linoleic acid (1, 49). Different nut types also differ in terms of micronutrients. For example, Brazil nuts are rich sources of selenium, whereas peanuts and hazelnuts provide high amounts of folate (48). Many nut types, especially almonds, hazelnuts, walnuts and pine nuts are good sources of vitamin E and are also inherently low in sodium (1).

Nuts are also rich in bioactive compounds called phytochemicals. The predominant phytochemicals in nuts are carotenoids, phenolic acids, polyphenols, phytosterols, phytates, lignans, hydrolysable tannins and naphthoquinones (49-51), although the phytochemical content of nuts varies considerably. Factors influencing this include, nut type, genotype, pre- and post-harvest conditions and storage conditions (50). The presence of a wide variety of phytochemicals and nutrients in nuts has been associated with anti-inflammatory, antioxidant, antiproliferative and hypocholesterolemic properties (49, 51). These favorable effects are likely due to the synergistic effect of bioactive compound in conjunction with the unique fatty acid profile found in nuts (49, 51, 52). However, some phytochemicals, such as phytate have also been considered as anti-nutrients, due to their ability to decrease mineral bioavailability (15).

2.3 Effect of nut consumption on diet quality

Recent epidemiological studies have investigated the relationship between nut consumption and diet quality (53-57). These studies have found that nut consumers consumed significantly higher amounts of energy (14-15%), total fat (12-23%), MUFA (17-30%), PUFA (22-43%), fibre (22-30%), vitamin A (11-23%), vitamin C (7-32%), vitamin E (39-48%), vitamin K (20-35%), folate (6-26%), iron (8-21%) , vitamin B6 (12-19%), thiamin (7-15%), niacin (7-19%), riboflavin (6-11%), calcium (8-16%),

magnesium (25-38%), zinc (7-23%), selenium (20%), phosphorus (9-20%) and copper (29-39%) compared with non-nut consumers (53, 55-57). Additionally, consumers also had lower intakes of sodium (7-9%), cholesterol (9%) and carbohydrates (4-10%) compared with non-nut consumers (53, 55-57). However, in one study analysis based on gender indicated diet quality significantly improved in male nut consumers compared with non-nut consumers whereas there were no statistically significant differences for women (54). This result could be due to frequency and portion size of nut consumption being higher in men than women in the study. Overall, studies have consistently shown improved diet quality among nut consumers compared to non-nut consumers. However, due to the cross-sectional nature of the studies, causal associations cannot be assumed. Intervention studies have also examined the changes in diet quality and the nutrient profiles with inclusion of nuts in the diet (13, 58-60). The findings from these studies show a significant increase in total fat (20-28%), MUFA (7-42%), PUFA (24%), vitamin E (17%), fibre (12%), magnesium (23%), and copper (15%) intakes with nut consumption compared to their control counterparts (13, 58, 59). Additionally, statistically significant decreases in CHO (10%), SFA (3%), Sodium (21%) and animal protein (9%) were also reported which are consistent with the epidemiological findings (13, 58, 59). Importantly, these positive changes in diet quality were observed in studies that included different nuts without the need of any additional dietary advice. Overall, these studies have shown that adding nuts into the usual diet results in higher intake of total fat, MUFA, PUFA, and vitamin E, along with lower intakes of CHO, SFA, and animal protein (13, 58, 59).

2.4 Phytate content of nuts

Phytate (a salt of phytic acid) is one of the predominant types of bioactive compounds in nuts (61). Phytic acid (also called myo-inositol hexa-phosphate or IP6) is found in abundance in plant derived foods such as legumes, cereals and nuts, and serves as a storage form of phosphorus (16, 62). Phytic acid formation occurs during maturation of the plant seed and can contain approximately 60-90% of total phosphate (61, 62). Phytic acid forms stable complexes with cations and are present as salts of calcium, magnesium or potassium and as mixed salts, called phytate (62, 63). Other than phytic acid, inositol penta-phosphates, inositol tetra-phosphates and inositol triphosphate (also called phytate) are present as well but in lower levels (<15%) (16). These inositol phosphates are the result of degraded phytic acid of foods during processing by the enzyme phytase (myo-inositol hexakisphosphate phosphohydrolase) (16, 26). These lower inositols have a lower binding ability with minerals and therefore are less influential on mineral bioavailability (26).

Phytic acid content in nuts can vary from ~0.1 – 9% (dw), with the highest phytate content found in almonds, Brazil nuts and walnuts (**Table 3**) (16). In comparison, the phytic acid content in cereals and legumes ranged from ~ 0.06 – 2.2% (dw) and ~0.2 – 2.9% (dw) respectively (16, 64). The range in phytate content across and within nut type not only reflect the different botanical varieties of nuts but also the environmental conditions, location, climate condition for optimal growth, soil type, fertilizer application, year of production/harvest, different maturation stages of nuts and storage (temperature and duration) (16, 50, 62, 65). Some variability in the literature is also a result of the different methods to measure inositol phosphates as well as the different forms measured.

Despite the variability in phytate content in different food, phytate continues to create controversies with both purported positive and negative effect on health. The literature indicates that phytate interferes with the bioavailability of some minerals and trace elements, however, recent studies have also shown beneficial effects of phytate (16, 61).

Table 3: Phytic acid/phytate content in 100g Nuts

Type of nuts	Phytic acid/phytate mg/100g (dw)[1,2]
Almonds	350 – 9420
Brazil nut	290 – 6340
Cashews	190 – 4980
Hazelnuts	230 – 920
Macadamias	150 – 2620
Peanuts	170 – 4470
Pecans	180 – 4520
Pine nuts	200
Pistachios	290 – 2830
Walnuts	200 – 6690

[1] Source: Schlemmer U, Frølich W, Prieto RM, Grases F. Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis (16)
[2] Depending on data published
Abbreviations: dw, Dry weight

2.5 Potential Benefits of phytate on health

Recently, phytate has been purported to have several beneficial effects on health (16, 61). Antioxidant and anti-cancer activities have mainly been reported, providing protection against colon and breast cancer and prevention of liver, prostate, pancreatic, rhabdomyosarcoma blood and bone marrow cancer in animal and invitro studies (16, 61). Additionally, phytate has been associated with the prevention of renal stone formation and renal lithiasis; reductions in risk of coronary heart disease (CHD) (lowers serum cholesterol and triglyceride levels), reduced incidence of fatty liver, reduced incidence of diabetes; improved hypolipidaemic activity; improved antiplatelet activity; protection against HIV and reduced risks of teeth decay/dental caries (16, 61).

However, to date there is limited research in this area, and studies conducted were either in animals or in-vitro studies, which limits the extent they can be extrapolated to humans. Further human studies are needed to evaluate the safety and clinical effectiveness of phytate and its beneficial effects on health.

2.6 Effects of phytate on nutrient bioavailability

Phytate is considered as an anti-nutrient as it interferes with the absorption of some minerals and trace elements (such as iron, calcium, magnesium and zinc) in the gut, which can increase the risk of micronutrient deficiencies, especially when intake of these nutrients are low (16). Although there are other anti-nutrients (tannins, cyanogenic glycosides, oxalates, saponins, lectins and enzyme inhibitors such as alpha-amylase, trypsin and chymotrypsin), phytic acid is the one most commonly found in cereals, legumes and nuts (15, 66).

Phytic acid has a highly negative charge density due to six negatively charged phosphate groups covalently bound to a small inositol molecule (16). The negatively charged oxygen atoms on adjacent phosphorus groups in phytate are arranged in such a way that positively charged cations can form tight bonds to each phytate group (16). Hence, phytic acid can form strong complexes with metal ions, particularly zinc, magnesium, calcium and iron. The binding of these ions results in formation of salts which are only soluble in acidic conditions (in the stomach), however under neutral pH (in the intestine) the complexes precipitate and become insoluble (and so cannot be digested by humans), leading to poor bioavailability and absorption of the micronutrients (16, 62). Furthermore, humans have limited ability to hydrolyse phytate molecules due to the lack of phytase enzymes, hence phytate phosphorus is not nutritionally available (63).

Myo-inositol hexa-phosphate (IP6) and inositol penta-phosphate (IP5) represent the main forms of phytic acid that interfere with trace elements and mineral bioavailability (15). The lower inositol phosphates (IP-4, 3, 2, 1) have less of a negative effect on bioavailability of minerals and trace elements, as they cannot form strong complexes (15). However, it seems that IP3 and IP4 along with IP5 and IP6 may have inhibitory effects on non-haem iron (67). Furthermore, the inhibitory effects of phytic acid on non-haem iron are dose dependent therefore establishing an ideal molar ratio is challenging, although, a molar ratio greater than 1 is considered inhibitory (63, 68-70). Inhibition of zinc absorption occurs when phytate:zinc ratio is 15 or above (70). Whereas, inhibition of calcium occurs when phytate:calcium ratio is 0.24 or above (70). Increased levels of calcium increase the effect of phytate on zinc absorption (61, 69). This is due to the formation of an insoluble calcium-phytate-zinc complex therefore phytate x calcium:zinc with a molar ratio greater than 200 would be a better indicator of zinc inhibition (68).

There is evidence that diets high in phytate significantly decrease the absorption of essential micronutrients (62). There are several factors that influence the inhibitory effect of phytate on minerals. These include the ratio of phytic acid to mineral, the presence of the type and amount of phytase enzyme, solubility of phytates (e.g. more soluble at lower pH), temperature (optimal range 45-57°C) and concentrations of enhancers, inhibitors as well as other minerals in the food (15, 16, 62, 71). Research to date suggests food processing methods are effective in phytate removal. However, it is important to consider these factors when choosing the type of food processing method to reduce phytate and increase mineral bioavailability.

2.7 Interventions to reduce phytate content of foods

Several methods such as milling, soaking, dry heating, fermentation, germination, cooking/ microwaving, adding exogenous enzymes or a combination of these have been used to remove phytate from food (61, 72). However, methods other than milling, soaking and dry heating are beyond the scope of this thesis and therefore will not be discussed extensively. In cereals, phytate is in the aleurone layer (~80% in small grains such as wheat and rice) and the germ whereas the endosperm contains no phytate (16). In legumes, phytate is primarily located in the protein bodies of the endosperm and cotyledon (~90%) (16). There is no literature to date on the location of phytate in nuts, therefore, there could be important differences in the effectiveness of processing methods in cereals and legumes compared to nuts. Studies suggest that antioxidants in nuts are predominantly in the pellicle (soft outer shell), which is an important consideration, because any processing that removes the nut skin can result in loss of antioxidants (1, 52, 73).

2.7.1 Milling/Particle size reduction

The process of reducing the size of grains, legumes and nuts are common practice in industries and at home (chopping/ slicing/ grinding). A recent study by Majzoobi et al. examined the effects of particle size reduction of wheat bran on phytic acid (24). Results showed a statistically significant reduction of 12.5-56.9% in phytic acid by reducing bran particle from 1,200µm to 90µm (24). Phytic acid reduction is likely due to the increase in surface area. However, it should be noted that reducing bran particle from 1200 to 90 µm was also associated with decreased levels of calcium (0.080 to 0.046 %), iron (0.026 to 0.016 %) and zinc (0.006 to 0.003%) (24). No other studies could be found that solely investigated particle size reductions, although, studies that

combined particle size reduction with other processing methods observed a reduction in phytic acid (33, 34). For example, when Perlas et al. soaked whole mung beans for 1 hour and 6 hours, no reduction was observed, however when mung bean flour was soaked, phytate content was reduced by 10% and 47% respectively (33).

Milling (removal of outer layer) is another way of reducing phytic acid in grains and legumes (16). When Sudanese sorghum was milled the reduction in phytic acid levels were mainly observed due to the removal of the outer layer of the grain where phytic acid was concentrated (32). Although this is efficient in reducing phytic acid and other anti-nutritional components, milling shows limited promise for improvement of mineral availability due to removal of minerals and dietary fiber in the process (24, 72). Furthermore, it appears that the degree of milling and particle size reduction have a greater effect on phytic acid reduction when combined with other methods of processing such as soaking, fermentation and germination (29).

2.7.2 Soaking

Soaking has been proposed as an easy and practical method to increase mineral availability in grains and legumes by reducing phytate content through hydrolysis of endogenous phytase, as well as passive diffusion into the soaking medium (25, 74). Soaking can be done as a pretreatment to other processing methods such as fermentation and germination, or independently for phytate removal (61).

To date only one study has examined the effects of soaking on the phytate content of nuts. Lin et al. reported an increase in phytic acid content when whole almonds were soaked in water for 15hr at 25°C, with higher levels when almonds were soaked at 40°C (75). However, no explanation for the increase in phytate was provided. The study procedures used were different to the current study (longer soaking time and lower

drying temperature). The authors also used an indirect method of phytate analysis, where all the phosphorus in the almonds were assumed to be from phytate. This is likely to overestimate the phytic acid content in the almonds, therefore, their results are not entirely comparable to the present study.

Numerous studies have investigated the effect of soaking on phytate content in whole and milled grains and legumes (25-34, 54). The extent of phytate reduction has varied when examining the effects of soaking on whole grains and legumes, whereas the results are more consistent when these are milled (33). For example, substantial phytate reduction was seen in whole sorghum and maize after soaking (26, 29). However, phytate reduction of ~ 19-29% was seen for rice, rye and triticles and 16-31% for African yamabean (26, 27). Similarly, soaking brown rice at 10°C after preheating reduced phytic acid by 42-59% (31). The differences in results can be due to variation between phytate profile, location of phytate in grain, phytate solubility, soaking duration, pH of soaking solution, degree of dehusking and dehulling, and any previous thermal treatment within cereals and legumes (61, 71, 72). Furthermore, variation in study protocols could also impact the varying results.

On the other hand, when milled sorghum and maize were soaked for 6–12 hours, greater phytate reduction (39% and 57%, respectively) was observed (29). Similarly, reductions in phytate were also observed in rice flour, wheat bran and quinoa flour (24, 33, 34). Interestingly, when pounded maize was soaked at room temperature for 1 hour, phytic acid reduction of 51% was observed as milled maize had a phytic acid reduction of 57% (76). Similarly, Hotz et al. reported phytic acid reductions of 51% in pounded maize (28).

Milling or pounding grains results in greater reductions of phytic acid. However, multiple studies have found that the effects of soaking milled grains may have adverse effects on mineral availability, which outweigh the beneficial effect of phytate reduction (26, 29, 30). This is likely due to the increased surface area of milled grains, where other nutrients along with phytate leach out into the soaking water, possibly leading to important losses of essential nutrients.

Hotz and Gibson et al. found that longer soaking time, higher volume of soaking liquid and proper removal of soaking liquid increases the amount of phytate that is lost (76). Studies that used a lower grain:soaking solution ratio showed less phytate content reduction, although, changing the soaking solution showed a greater reduction (76, 77). It has also been shown that phytate hydrolysis increases when exposed to optimal temperature (45-65 °C) and pH (5 and 6) during soaking (29, 61, 72). Hence, these are all important factors to consider when soaking cereals and legumes. However, the effects of soaking on nuts are currently unknown.

2.7.3 Dry heat treatment

Dry heating as a treatment has produced mixed results on phytic acid content. In one study dry heating (roasting) decreased phytic acid content in legumes (65), and a further decrease was observed when both the temperature and duration of heating were increased compared to control condition (no heating) (78). On the other hand, some studies have found heat had no effect on phytate content in legumes such as field peas, chick peas, faba beans and African yambeans (27, 79). Furthermore, Arinola et al. showed an increase in phytate content when walnuts with shells were roasted in sand for 1hr (80). The differing study results may be due to different exposure time, temperature, difference in heating procedure, and failure to consider potential moisture loss.

2.7.4 Fermentation

Fermentation is effective in reducing phytic acid through microbial and/or enzymatic methods (hydrolysis of endogenous phytase) in both cereals and legumes (24, 27, 31, 34, 81). The results are augmented when combined with particle reduction or the addition of an enrichment starter (24, 31). Interestingly the differences in phytase enzyme and enzyme activity influence the degree of phytic acid reduction (16). However, due to the acidic nature of the processing method the acceptability of these processed cereals and legumes are questionable. In addition, research needs to assess the effectiveness of this method of reducing phytate in nuts, and indeed whether the acceptability of nuts with consumers is affected.

2.7.5 Germination

Germination of grains and legumes significantly reduces phytic acid through degradation by endogenous phytase where the grain utilizes phytate as a source of inorganic phosphate in the germination process (26, 61, 82, 83). Liang et al. highlighted that during steeping, phytic acid and minerals leach out in to the water (mainly in cereals) (31). However, during sprouting, the phytase enzyme hydrolyses phytic acid into inorganic phosphates and inositols in cereals and legumes which contain high phytic acid content in the endosperm (31, 32). Additionally, when germination and fermentation were combined near complete degradation of phytate was achieved (34).

2.7.6 Summary of phytate reduction methods

Overall, studies to date have examined various methods of phytate reduction in cereals and legumes with varying results. Soaking is more effective in reducing phytate in milled cereals and legumes rather than whole because of an increase in surface area. However, when whole cereals and legumes are soaked, phytic acid removal is more

effective in cereals than in legumes, where variability in phytate distribution plays an important role. Microwave, dry and wet heating have also been shown to reduce phytic acid; where temperature, period of heating and particle size are all important factors to consider when using these methods (24, 78, 84). However, methods involving enzymes for phytic acid removal were found to be more effective than physical extraction methods, i.e. milling, soaking and heating; where germination was more effective than fermentation. This is due to the ability of endogenous phytase to break down phytate within the cereal and legume and use it during the germination process. However, this process is limited in how it can be extrapolated to nuts due to different chemical composition of nuts compared to cereals and legumes.

2.8 Methods described in the lay literature for reducing the phytate content in nuts

There are numerous reports in the lay literature advocating different methods of 'activating' nuts to provide maximal health benefits. The term 'activating' refers to neutralization of enzymes inhibitors present in nuts, hence, allowing greater nutrient bioavailability, proper digestion and changes in texture and flavor of the nut (17-21). While there is a vast amount of information in lay literature on 'activating' nuts, there is no consensus on the best method. However, there are some common practices seen in recommendations. In general, the soaking time is influenced by how hard the nut is. For example, nuts such as almonds and hazelnuts are recommended to be soaked for a longer time compared to walnut and cashews which are softer. Salt is often suggested as an additive to the soaking medium. It is purported that salt is added in order to activate enzymes that are responsible for deactivating the enzyme inhibitors in nuts (19, 20, 23). The different 'activating' methods are outlined in **Table 4.**

Table 4: Different methods of 'activating' nuts

Method	Nut type	Procedure
Soaking (21, 85)	Any nuts and seeds	Cover nuts with warm water. Soak in a warm place for 18 hours (drain, rinse and add new water half way) Dehydrate at a very low temperature either in an oven or a food dehydrator. Then roast in the oven or on the stove.
Soaked in salt (17-19, 86, 87)	Almond, Brazil nuts, Cashews, Pecans, pine nut, peanut, pistachios, hazelnut, macadamia, walnuts	4 cups of nuts 1 Tbsp unrefined sea salt (For almond, cashews, peanut- skinless, pine nut, hazelnut-skinless, macadamia nuts) Or 2 tsp unrefined sea salt (Pecans, walnuts) Similarly, salt was halved for pecan and walnut compared to any other nut type (22) Filtered water (enough to cover nuts) or 1-part nut:2 parts water. Soak based on the allocated hours for each nut type Rinse thoroughly and dehydrate for 12-24 hours or until crisp (do not use temperature above 65°C) (20) Place them in sealed glass jars and store them. (table continued next page)

		Soaking time: Almonds: 8-12 hours (23, 86, 87); 7+ hours, maximum 24hrs (17, 19, 88) Brazil nuts: 3-8 hours (87) Cashews: 2-4 hours (87, 89); maximum of 6 hours (19, 23, 88); minimum of 7 hours suggested in one method (17) Hazelnuts: 8-12 hours (17, 19, 23, 87) Macadamia nuts: 8-12 hours (17, 19); 2 hours suggested in one recipe (87) Peanuts: 7+ hours, maximum 24hrs (19, 88) Pecans: 4-6 hours (87); 7 hours (17, 19, 88); 8-12 hours (23); 4-8 hours (90) Pine nuts: 7+ hours (17, 19) Pistachio: 4-8 hours (87) Walnuts: 4-8 hours (19, 87, 91); 8-12 hours (17, 23)
Sprouting/ Germination (87)	Almond	1) Place soaked and rinsed nuts in a jar and cover with the lid or cloth. 2) Lay the jar in an angle on a sunny window seal to allow the excess water to drain, and leave it to sit in the light 3) Every 8hrs, thoroughly rinse the contents of the jar (making sure you get all the water out each time). 4) Keep the jar in the sunlight when your nuts start to sprout and continue the process until fully sprouted. 5) Once completely dry when touched, store sprouts in the fridge (will keep in the fridge for 2-3 days) Almonds: 3 days maximum, other nuts were suggested not to be sprouted (87).
Fermentation in whey solution (88)	Any	Place 4 cups of nuts in a bowl, cover with water and ½ cup whey. Soak for 24 hours and dehydrate for 12-24 hours or until dry.

Abbreviations: Tbsp, tablespoon; tsp, teaspoon

2.9 Overall summary

Nuts are a nutritionally rich food, where regular consumption has consistently been associated with lower all-cause mortality and CVD (2-11). However, recently anti-nutritional components within nuts have received a lot of attention, particularly in the lay literature, regarding mineral bioavailability. Phytate is one of the main anti-nutrients that affects the bioavailability of minerals such as iron, zinc, magnesium and calcium when consumed in high amounts. This is primarily important if the intake of these minerals are low or if an individual has a vegetarian or vegan diet where consumption of phytate containing foods are higher. The literature suggests a number of methods of food processing can reduce phytic acid concentrations in grains and legumes, although, only a few studies have looked at the effect on nuts (80, 92). Recently, claims in the lay media have advocated the need to 'activate' nuts to maximize the nutritional benefits. However, there is no research to date to support this practice and the knowledge around the effect of soaking nuts on phytate and mineral concentrations is still unclear.

3 Objective Statement

Nuts are nutrient dense and are well known for their numerous health benefits. Currently Ministry of Health dietary guidelines recommend people consume 30g of nuts daily for a heart healthy diet. However, many recent reports in the lay literature have promoted the 'activation' of nuts for optimal health benefits. Advocates of nut activation claim soaking decreases phytate, a compound which inhibits mineral absorption. However, soaking may also result in the leaching of minerals and water-soluble vitamins e.g. folate. Currently there is no evidence to support or refute claims about 'activating' nuts. Research examining the effects of soaking nuts on phytate and micronutrient concentrations is required to inform evidence-based guidelines, and hence messages to the public, regarding the optimisation of the overall nutritional value of nuts.

The overall aim of the study was to assess the effect of soaking almonds and hazelnuts on phytate and mineral concentrations (iron, zinc, magnesium, potassium, phosphorus, calcium and sodium).

The specific objectives include to:

1. Examine the effect of soaking different forms (whole and chopped) of almonds and hazelnuts on phytate and mineral concentration.
2. Examine the effect of soaking duration (12hrs vs 4hrs) on phytate and mineral concentration.
3. Assess whether the addition of salt to the soaking medium has any effect on phytate and mineral concentration.
4. Assess the impact of adding salt to soaking solution and the concentration of sodium in the nuts.

4 Method

4.1 Study design

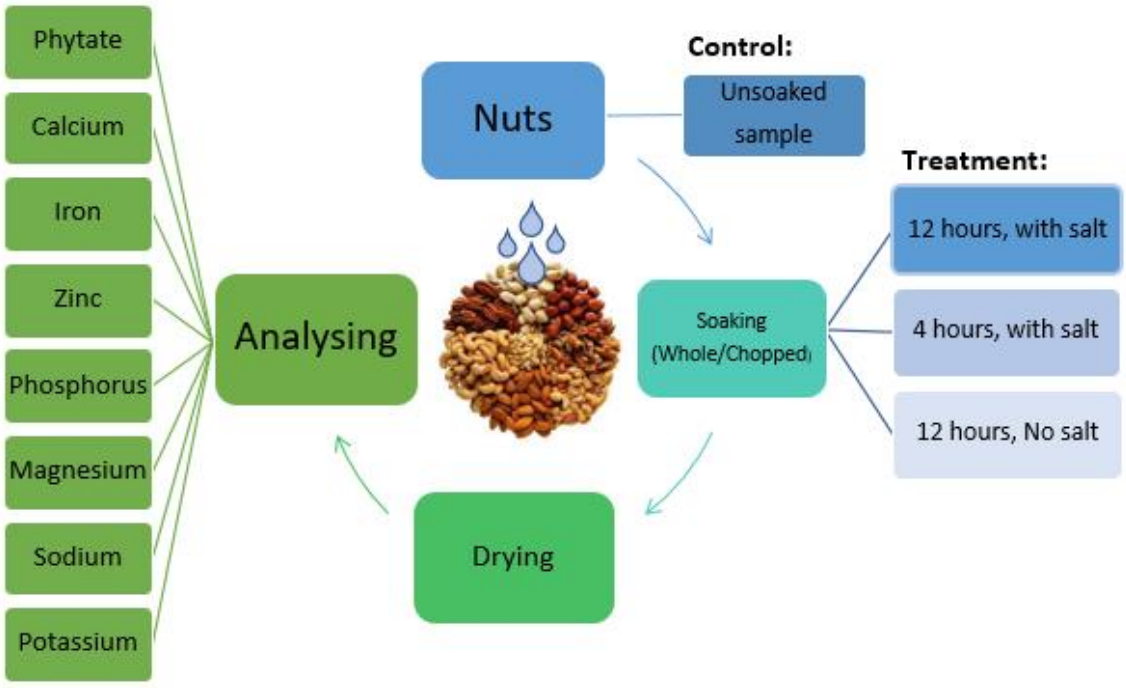

Figure 1: The SNAP study experiment overview

The Soaking Nuts And Phytate (SNAP) study assessed four popularly consumed nuts in New Zealand, Europe, and the USA (14, 54, 93). Four nut types: almonds, hazelnuts, peanuts, and walnuts (referred to as 'nut type' hence forth), and two different forms of each nut were analysed: whole and chopped (referred to as 'nut form' from now onwards). Each type- form combination (seven in total – untreated raw served as the control treatment for both whole and chopped nuts) underwent each of the four soaking treatments outlined in **Figure 1** (Unsoaked, 12hr+salt, 4hr+salt and 12hr-salt). This study was conducted as an incomplete factorial design, where the unsoaked (untreated) nuts were all in the whole form only, not chopped. This was because the method for

analysing the outcomes, such as phytate, required the nut samples to be ground before analysis. As all nuts types were purchased whole, it was not considered necessary to first chop the raw untreated nuts, for them to be immediately ground, ready for analysis (i.e. the results would be the same for whole and chopped forms given that there was no storage period or de-skinning involved). Additionally, the sample size required five replicates. These were obtained by purchasing five different brands for each nut type and using each as a replicate (rather than homogenizing the nuts from the different brands) to enhance the generalisability of the results **(Table 5)**. This meant there were 35 treatments per nut type and a total of 140 samples were analysed **(Figure 2)**. However, for the purpose of this thesis only the results of almonds and hazelnuts will be reported.

4.2 Study nuts

All nuts were purchased from either supermarkets or local Farmer's markets in Dunedin, New Zealand. All the nuts were purchased as whole and raw. The nuts were purchased between February and March 2017 and stored in a cool, dark place in a sealed packet until processed and analysed, **Table 5** shows the brands of each nut type purchased.

Table 5: Brands of nuts purchased

Nut	**Brand 1**	**Brand 2**	**Brand 3**	**Brand 4**	**Brand 5**
Almond	Tasti	Freshlife	Sun Valley	Pams	Mother Earth
Hazelnut	Tasti	Pams	Freshlife	Amazelnuts*	Marlborough
Peanut	Gilmours	Freshlife	Budget	Sun Valley	Tasti
Walnut	Tasti	Marlborough	Macro	Freshlife	Pams

*Nuts purchased from the local Farmers market

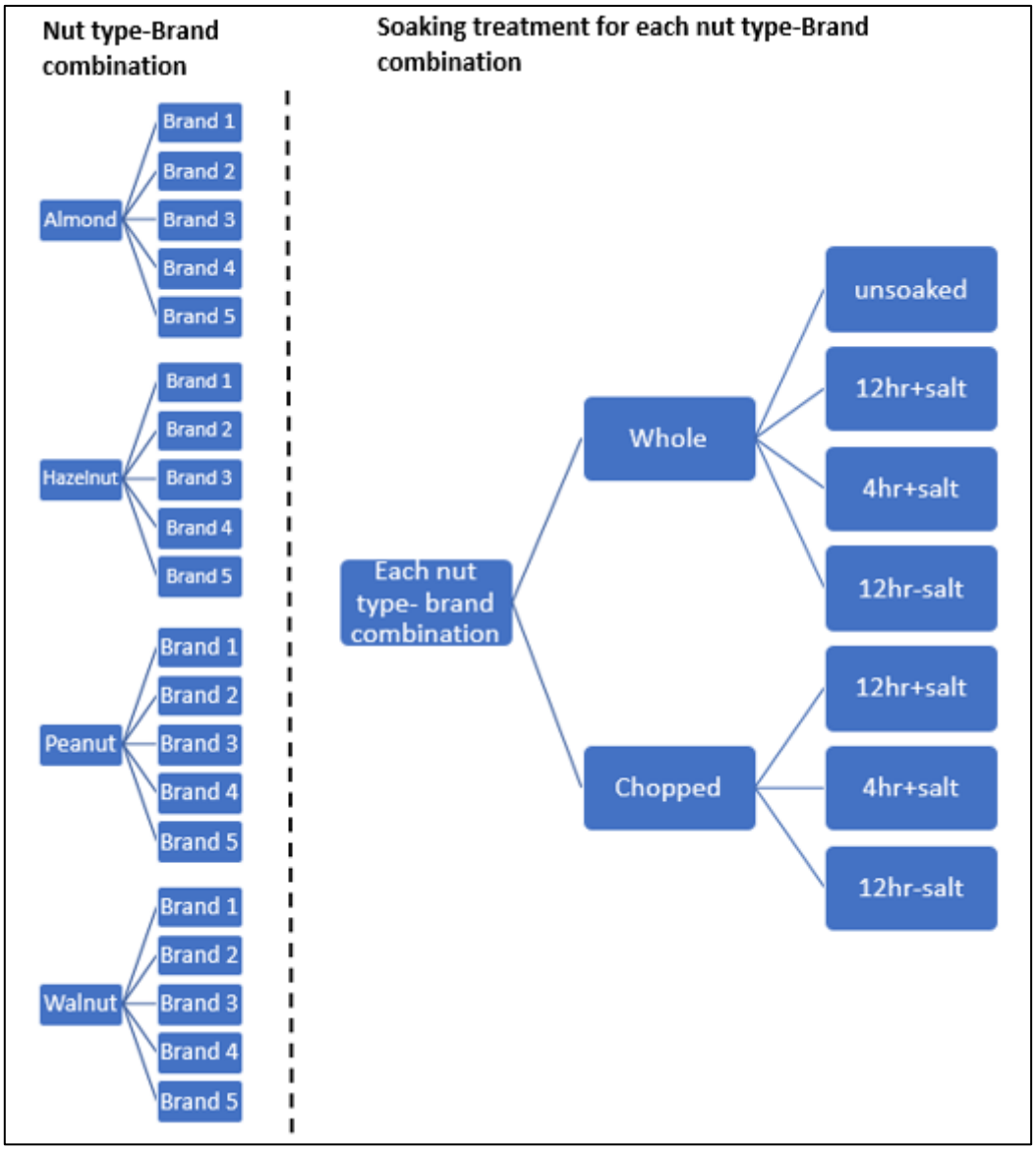

Figure 2: Soaking treatment for each nut type-brand-form combination

4.3 Preparation of chopped nuts

To obtain chopped nuts, 140 g of nuts were weighed for each treatment group (3 chopping batches for each brand of nuts). The nuts were then chopped in a food processor (Robot Coupe R211 Ultra) with a blade attachment for an allocated time outlined below. These allocated times were trialled to obtain uniformly chopped nuts between nut types (time was dependent on the hardness of the nut).

Chopping time for each nut:

- Almond – 10 seconds
- Hazelnuts – 5 seconds
- Peanut – 5 seconds
- Walnut – 5 seconds

The chopped nuts were then put through a sieve (aperture 1.18 mm) to remove all the finely chopped pieces. The advantage of controlling the chopping time as well as sieving the nuts ensured the samples were uniformly chopped within the chopping batches and across the different brands for each nut type. The chopped nuts were then stored in a food grade air tight container in a cool dark place.

4.4 Soaking protocol

The soaking protocol was established from popularly reported methods in the lay literature (20, 22, 23). This was then trialled prior to conducting the study to assess its feasibility. The soaking protocol contained four soaking treatments, a control (unsoaked) and three soaking treatment groups. Specific procedures for soaking in each treatment groups are described below:

(1) Unsoaked nuts (Control) – Raw nuts, no soaking or drying. This untreated sample was considered a control for both the whole and chopped nuts because all samples were ground and analysed within 2 days and skins were retained therefore no differences would have been expected for whole untreated and chopped untreated forms of the nuts.

(2) Original 12 hours soaking – 100 g of raw nuts were soaked for 12 hours in 240 g of Millipore water and 5 g of salt (Cerebos plain table salt, Dunedin, New Zealand)

(3) 4 hours soaking – 100 g of raw nuts were soaked for 4 hours in 240 g of Millipore water and 5 g of salt.

(4) Salt-free 12 hours soaking – 100 g of raw nuts were soaked for 12 hours in 240 g of Millipore water and no added salt.

Approximately 100 g of either whole or chopped nuts were weighed into a food grade container. The nuts were soaked in 240 g water (equivalent to 1 cup); this ensured the nuts were fully immersed in water, in addition, all measurements were done in grams for accuracy and consistency. The nuts were then either soaked for 12 hours (with or without salt ~5 g) or 4 hours (with salt) at room temperature. Two different soaking times were examined to allow a deeper insight on the effect of soaking duration on phytate, and to see if soaking for shorter periods has any beneficial effect on reducing the amount of minerals leaching into the soaking solution. Salt was added to the soaking solution as it is claimed in the lay literature that salt aids in activating enzymes that are responsible for deactivating the enzyme inhibitors in the nut (19, 20, 23).

After soaking, the solutions were drained (not rinsed with water) and nuts were spread in a single layer on a baking tray and placed in an oven (Binder GmbH 115FD, Germany) to dry at 65°C for 24 hours. The weights for each sample were recorded before soaking and after drying. The nuts were then cooled and ground in a blender (Waring commercial blender 32B-80, USA). Control samples that did not undergo soaking treatment were also blended and stored in a cool dark place until further analysis.

4.5 Phytate analysis

The phytate content of the nuts was analysed using high-performance liquid chromatography (HPLC) using a method by Lehrfield (1989) with modifications (94). A brief summary of the method is as follows.

The Ground nuts (0.5 g) were soaked with 5.0 mL of 0.67M Hydrochloric acid (HCl). The sample was vortexed (IKA MS3 vortex machine, USA) for 2.5 minutes; placed in a Sonicator bath (Elma Transonic T890/H, Germany) for 30 minutes and vortexed again for 1 minute. These three steps dissolve any phytates in the nuts into the aqueous medium. The sample was then centrifuged for 15 minutes at 3200 RPM (Jouan C312, Cedex, France) to separate the supernatant from the Ground nuts. 2.5 mL of supernatant and 22.5 mL of water was added to 50 mL tubes ready to be placed in ion exchange columns (Sep-Pak Vac 1cc waters Acell^{TM} Plus QMA). A vacuum manifold was setup by attaching an ion exchange column and a 25 mL syringe barrel to the luer lock. The column was conditioned with 3 mL of 0.067 M HCl (flow rate approximately 1 mL/minute, vacuum at approximately 10kPa). The sample was then loaded into the syringe barrel, with a starting vacuum pressure of 10 kPa, which was increased when necessary (e.g. blockage/ when flow rate decreased) until all phytate was loaded into the column packing. The vacuum manifold was setup again to collect the sample eluant into labelled, 5 mL Nalgene containers. The phytates were then eluted off the columns using 4 mL of 2 M HCl, with a vacuum pressure starting at 10 kPa. The eluants were evaporated until completely dried on a heating block (at 60°C) in the fumehood. Once completely dry, the samples were re-dissolved in 1 mL of Millipore water before HPLC analysis.

The HPLC mobile phase consisted of 200 mL Millipore water, 2.5 mg dodecasodium phytate, 1.10 mL of concentrated phosphoric acid, 10 mL tetrabutylammonium hydroxide solution and 300 mL Hipersolv methanol with pH adjusted to 4.0 using 9N Sulphuric acid. Finally, the mobile phase was filtered using 0.45 µm nylon filter. The analysis was performed on a HPLC system installed with a refractive index detector (Agilent Infinity 1260 Series, USA) and a Hichrom (UK) Hypersil H3ODS 4.6x250 mm Analytic column (pore size 120Å). Samples were analysed in duplicate and results expressed as the sum of IP5 and IP6 in mg/100g. A pooled wheat bran sample was used to determine the precision of the HPLC methods. The phytate in the wheat bran was measured with each batch of 10 nut samples and gave an overall value of 10.7 mg/g with an inter assay coefficient variation (CV) of 7.0%.

4.6 Mineral Analysis

4.6.1 Digestion

Samples of 0.25 g of the homogenized nuts were weighed to ±0.001 g into a CEM 75 mL PFA microwave digestion vessel. A total of 5 mL of high purity nitric acid and 1 mL of high purity hydrogen peroxide was added and left to predigest for 30 minutes prior to capping. Forty vessels at a time were loaded into a rotor and a standard digestion program run in a CEM MARS 6 Microwave Digestion system. After cooling, the vessels vented and the digestate rinsed out with >18.2MΩcm water and made up to 50 mL in pre-weighed Digitubes (SCP Science, Quebec, Canada). Final volume was calculated by weight and density.

4.6.2 Minerals measurement

A 1.0 mL aliquot of the digestion solution was further diluted with 1.0 mL of 2% v/vHNO_3 and presented for dissolved metals analysis on an Agilent (Santa Clara, CA,

USA) 7900 Inductively coupled plasma mass spectrometry (ICP-MS). Multi-element calibration solutions were prepared gravimetrically from NIST traceable standard solutions High-Purity Standards (Charleston, SC, USA). A cocktail of reference elements not present in the samples was added online to enable correction of any changes in instrument response due to matrix effects or sample uptake issues. The instrument was tuned according to manufacturer's guidelines for general purpose samples with a range of elements determined in addition to the main nutrient elements. Several nut samples were digested in duplicate to establish whole digestion precision while several samples had repeated measurements to confirm measurement precision. Concentrations in nuts were calculated using the solution and nut weights and include a correction for mass loss on drying applied so as to report all results on an "as purchased" basis.

4.7 Derivation of molar ratios of phytate: calcium, phytate: iron, and phytate: zinc

The phytate to calcium, iron, and zinc ratios were calculated using the equation below.

$$\text{phytate :mineral molar ratio} = \frac{(phytate\ (mg) \div phytate\ molecular\ weight)}{(mineral\ (mg) \div mineral\ atomic\ weight)}$$

The phytate molecular weight used was 660 g/mol, and the atomic weight used for calcium,40.08; iron,55.847; and zinc, 65.37 (69, 95).

4.8 Sample size

To provide 80% power to detect a 70% reduction in phytate (a level that would be required in order to recommend soaking) with in any soaking group would require n=3 within that group based on a standard deviation for this of 12% (estimated from data provided in Holtz and Gibson,2001) (76). To detect a 25% difference in phytate reduction between any two treatments (again, the smallest difference we would find

useful for distinguishing between approaches) with the same power and level of significance, n=5 per group would be needed. The larger of the required sample size was used.

4.9 Statistical analysis

All outcomes of interest were described using appropriate summary statistics. Phytate and mineral concentrations and molar ratios were compared between experimental groups within each nut type using linear mixed models with a random "batch" effect and including an interaction term between the form (whole and chopped) and treatment (unsoaked, soaked 4 hours with salt, soaked 12 hours with salt, and soaked 12 hours without salt). The design was an incomplete factorial design with the unsoaked nuts all in the whole form and not chopped. Wald tests were used to assess overall evidence of differences between the seven experimental groups and pairwise comparisons were only performed when this overall test was significant. Log-transformations were used when this improved the satisfaction of model assumptions around residual normality and homoscedasticity. Stata 14.2 (StataCorp, College Station, Tx, USA) was used for the mixed model analyses and all tests were performed using a two-sided 0.05 level to indicate statistical significance.

5 Results

5.1 Introduction

This study was a collaborative project, hence, for the purpose of this thesis only the results of almonds and hazelnuts will be presented in this chapter. This study was conducted with three soaking treatments and with two different forms of nuts (whole and chopped); 1. whole and chopped nuts soaked for 12 hours in salt solution (12hr+salt), 2. whole and chopped nuts soaked for 4 hours in salt solution (4hr+salt), and 3. whole and chopped nuts soaked for 12 hours in water with no added salt (12hr-salt). These were compared to unsoaked whole nuts, as only the whole nut form was used as unsoaked control, there were a total of seven treatment groups which were compared against each other. The primary comparisons of interest for each nut type being between the whole unsoaked nuts and the six treatments and between the whole and chopped forms for each of the three soaking regimens. Each nut type analysed consisted of five different brands and therefore the results reported reflect the mean of the five brands as described in the methods. Furthermore, phytate (sum of inositol penta-phosphate (IP5) and inositol hexa-phosphate (IP6)) and seven different minerals (calcium, magnesium, phosphorus, potassium, iron, sodium, zinc) were assessed in both the untreated and treated arms for almonds and hazelnuts; the results were reported as mg per 100g.

Table 6: Mean (95% CI) phytate and mineral content of almonds for the different treatments[1]

Nutrient	Untreated	Soaked 12h+salt, chopped	Soaked 12h+salt, whole	Soaked 4h+salt, chopped	Soaked 4h+salt, whole	Soaked 12h-salt, chopped	Soaked12h-salt, whole	Overall p-value
Phytate (mg/100g)	531 (506, 556) ^{AB}	508 (483, 533) ^{A}	550 (524, 575) ^{BC}	539 (514, 564) ^{ABC}	571 (546, 596) ^{C}	515 (490, 541) ^{A}	559 (533, 584) ^{BC}	**0.001**
Calcium (mg/100g)	253 (232, 273) ^{A}	230 (211, 249) ^{B}	234 (215, 253) ^{B}	230 (211, 249) ^{B}	242 (222, 262) ^{AB}	235 (215, 254) ^{B}	254 (233, 275) ^{A}	**<0.001**
Iron (mg/100g)	3.5 (3.2, 3.8) ^{A}	3.0 (2.7, 3.3) ^{B}	3.4 (3.1, 3.7) ^{A}	3.0 (2.7, 3.3) ^{B}	3.5 (3.2, 3.8) ^{A}	3.0 (2.7, 3.3) ^{B}	3.3 (3.1, 3.6) ^{A}	**<0.001**
Magnesium (mg/100g)	264 (251, 277) ^{A}	224 (213, 235) ^{B}	248 (236, 260) ^{C}	223 (212, 234) ^{B}	250 (238, 262) ^{AC}	233 (222, 244) ^{B}	257 (244, 270) ^{AC}	**<0.001**
Phosphorous (mg/100g)	468 (449, 487) ^{A}	429 (410, 448) ^{BC}	452 (433, 470) ^{AC}	426 (407, 445) ^{B}	456 (437, 475) ^{A}	420 (401, 439) ^{B}	460 (441, 479) ^{A}	**<0.001**
Potassium (mg/100g)	673 (616, 731) ^{A}	418 (361, 476) ^{B}	588 (531, 645) ^{C}	451 (394, 509) ^{B}	625 (567, 682) ^{CD}	505 (447, 562) ^{E}	636 (579, 693) ^{AD}	**<0.001**
Sodium (mg/100g)	0.8 (0.3, 1.3) ^{A}	653 (219, 1086) ^{B}	182 (61, 303) ^{C}	578 (194, 962) ^{B}	141 (47, 235) ^{C}	3.9 (1.3, 6.5) ^{D}	1.5 (0.5, 2.5) ^{A}	**<0.001**
Zinc (mg/100g)	2.9 (2.8, 3.1) ^{A}	2.7 (2.6, 2.9) ^{BC}	2.8 (2.7, 3.0) ^{AC}	2.7 (2.6, 2.9) ^{BC}	2.9 (2.8, 3.1) ^{A}	2.7 (2.5, 2.8) ^{B}	2.9 (2.7, 3.0) ^{A}	**<0.001**

[1] Values with different superscript letters are statistically significantly different P<0.05

5.2 Phytate content in almonds

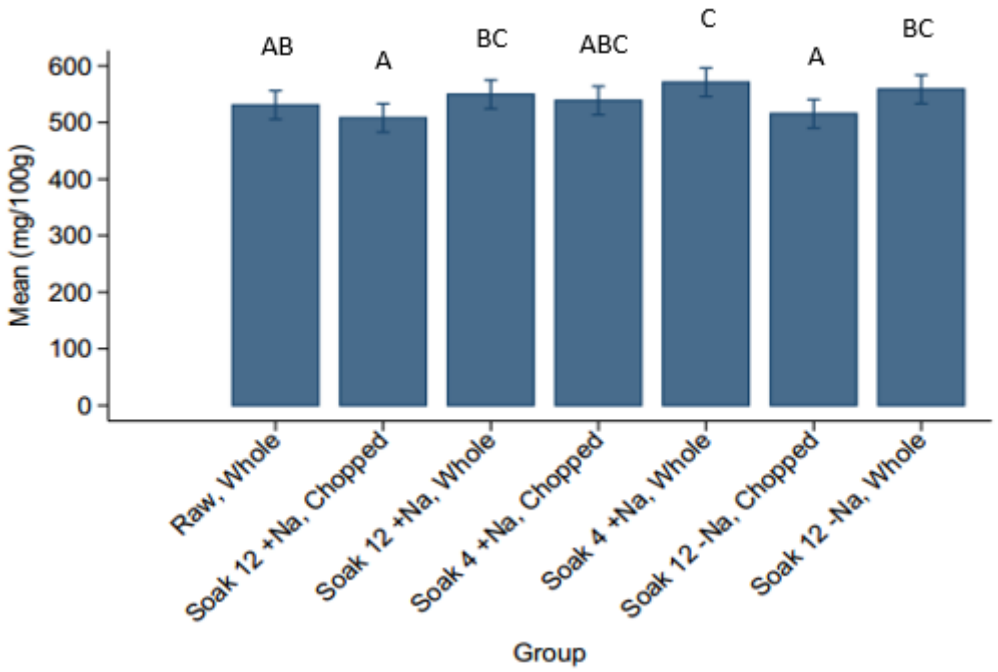

Figure 3: The mean phytate content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The untreated almonds had a mean (95% CI) phytate content of 531 mg/100g (506, 556), with the phytate content of the treated almonds ranging from 508 to 571 mg/100g **(Table 6)**. There were overall statistically significant differences between the treatments (overall p<0.001) **(Figure 3)**. When looking at the pairwise comparisons, compared to the untreated almonds, the only statistically significantly different phytate content was 4hr+salt whole which was higher with a difference of 40 mg/100g (p=0.015). The almonds which were chopped and soaked for 12 hours with or without salt had the lowest phytate content, but these values were only significantly lower compared to all whole treated nuts (all p≤.0.039). Further analysis showed 4hr+salt was the only soaking treatment that had no statistically significant difference between whole and

chopped almonds. Additionally, no statistically significant differences were observed across all the whole almond treatments ($p \geq 0.198$) and across all the chopped treatments ($p \geq 0.064$).

5.3 Mineral content in Almonds

5.3.1 Calcium

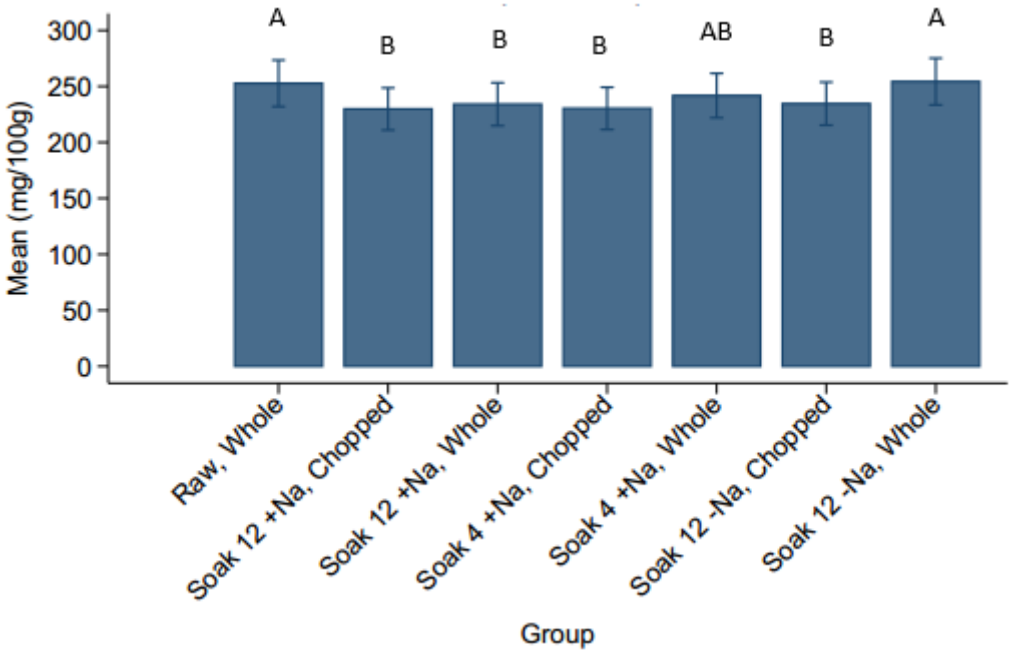

Figure 4: The mean calcium content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different $P<0.05$.

The calcium content for untreated almonds was 253 mg/100g. In comparison, calcium in whole treated almonds ranged from 234-254 mg/100g, whereas chopped treated ranged from 230-235 mg/100g **(Table 6)**. All whole treated almonds were not statistically significantly different compared to untreated almonds, except for 12hr+salt whole almonds, which was significantly lower ($p=0.003$) **(Figure 4)**. However, all

chopped almonds were statistically significantly lower than untreated almonds (all p≤0.004). Although, when all chopped almonds were compared to whole almonds for each soaking treatment, only 12hr-salt was significantly lower. Additionally, a significant difference was also observed between 12hr+salt whole and 12hr-salt whole where the latter was significantly higher (p=0.001).

5.3.2 Iron

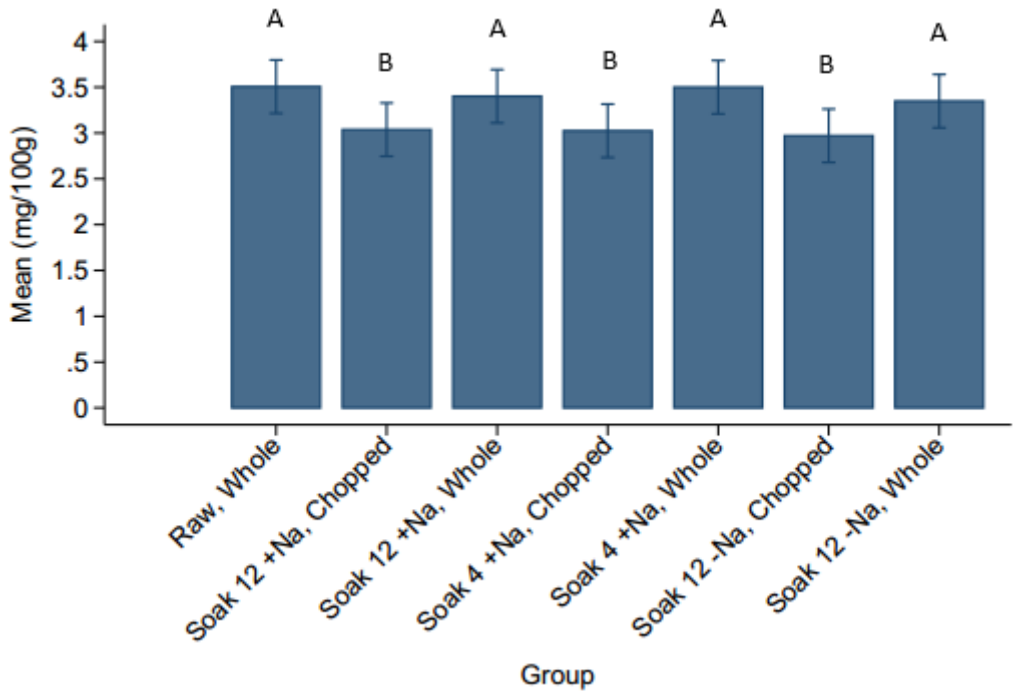

Figure 5: The mean iron content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

No statistical differences were found between any of the treated whole almonds (range of 3.3-3.5 mg/100g) and untreated almonds (3.5 mg/100g) **(Table 6) (Figure 5)**. However, a significant decrease in iron content was observed for all chopped treated almonds compared to untreated almonds (all p<0.001). There were also statistically significant differences observed between all chopped soaked treatments and their

corresponding whole soaked treatments where the iron content for chopped treatments were significantly lower than whole treated almonds (all p<0.001). Soaking duration and addition of salt to soaking solution was not associated with iron, with no differences across all the whole soaked treatments (p≥0.287) or across all the chopped soaked treatments (p≥0.474).

5.3.3 Magnesium

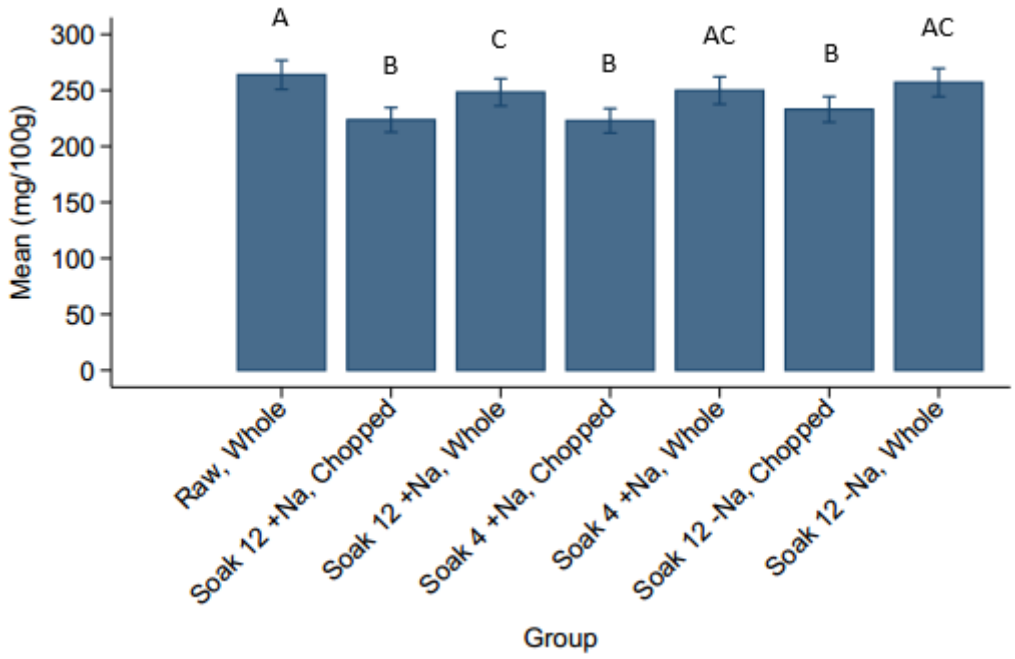

Figure 6: The mean magnesium content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The magnesium concentration for untreated almonds was 264 mg/100g whereas treated almonds ranged from 248-257 mg/100g in whole almonds and 223-233 mg/100g in chopped almonds **(Table 6)**. All chopped treated almonds had a significantly lower magnesium content compared to the untreated almonds (all p<0.001) **(Figure 6)**. However, for the whole almonds, only 12hr+salt was found to be statistically lower when compared to untreated almonds (p=0.047). Furthermore, all chopped soaked

treatments were significantly lower than the corresponding whole soaked treatments ($p \leq 0.001$). No differences were observed across all the whole soaked treatments ($p \geq 0.259$) and across all the chopped soaked treatments ($p \geq 0.178$).

5.3.4 Phosphorus

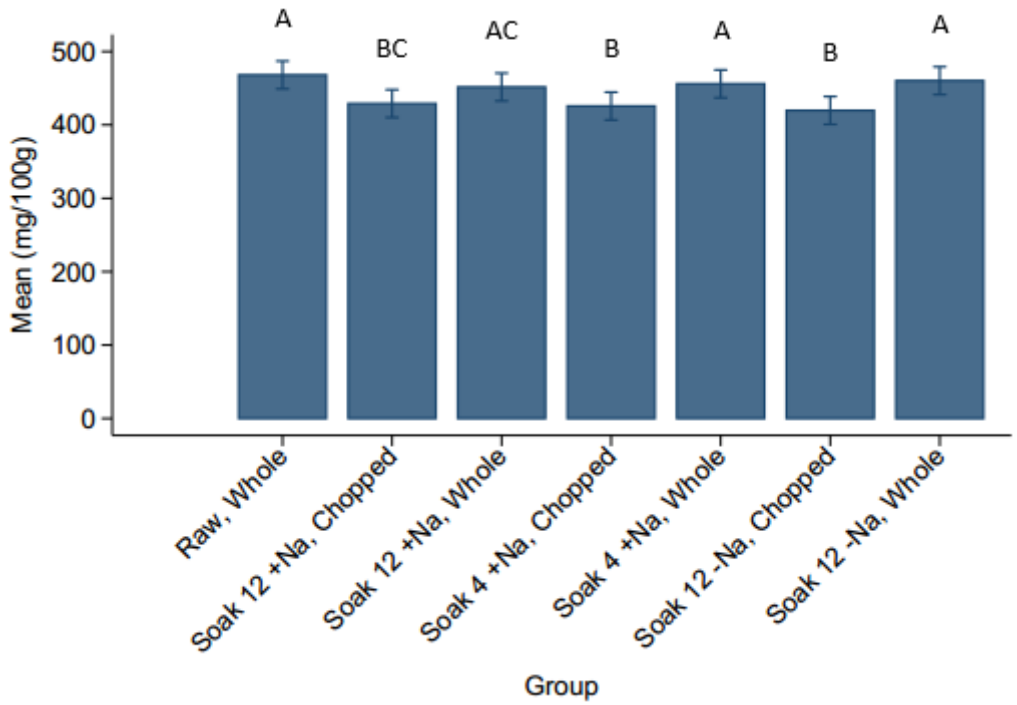

Figure 7: The mean phosphorus content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different $P<0.05$.

The phosphorus content for untreated almonds was 468 mg/100g, ranging from 452-460 mg/100g in whole treated and 420-429 mg/100g in chopped treated almonds **(Table 6)**. There was a significant reduction in all the chopped treated almonds compared to untreated group (all $p \leq 0.001$) **(Figure 7)**. However, no statistically significant differences were observed for whole treated almonds when the same comparison was made. Further, for two treatments (4hr+salt and 12hr-salt) chopped almonds had a significantly lower phosphorus content compared to whole almonds from the soaking treatment (both $p \leq 0.009$). There were no statistically significant differences when

comparisons were made across all the whole almond treatments (p≥0.457) and across all chopped almond treatments (p≥0.420).

5.3.5 Potassium

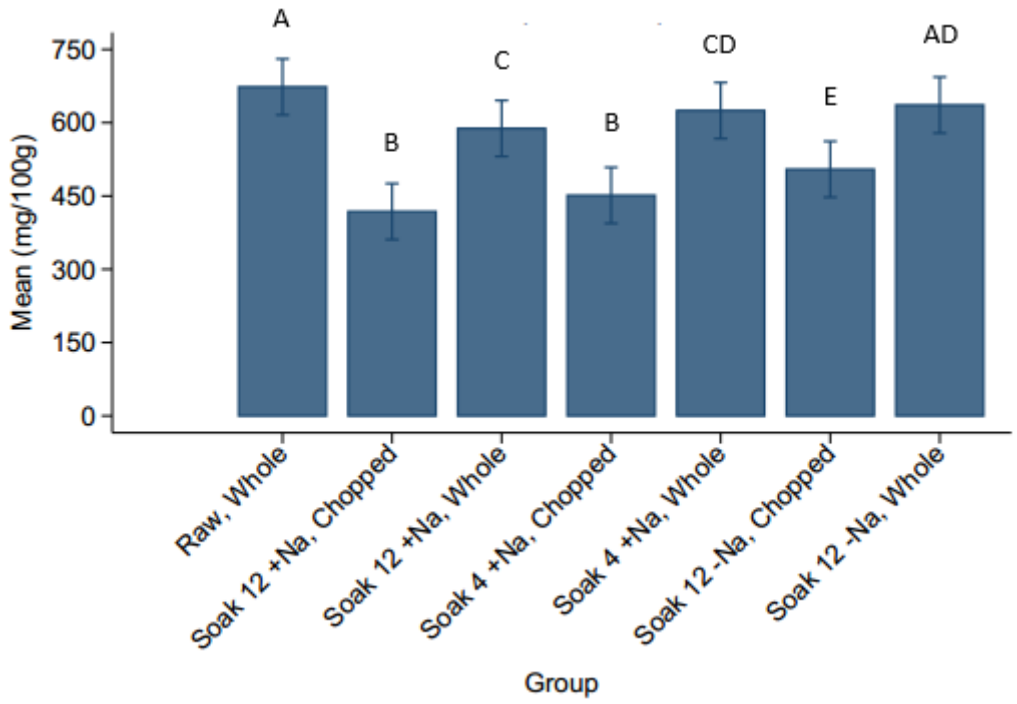

Figure 8: The mean potassium content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The untreated almond had a mean potassium content of 673 mg/100g; whereas the potassium in treated whole almonds ranged from 588-636 mg/ 100g and 418-505 mg/100g for treated chopped almonds **(Table 6)**. The potassium content of all the treated almonds were significantly lower compared to untreated almonds (all p≤0.019), except for 12hr-salt whole (p= 0.072) **(Figure 8)**. There was no statistically significant difference between 12hr+salt chopped and 4hr+salt chopped, however, both were significantly lower than 12hr+salt whole, 4hr+salt whole, 12hr-salt whole and 12hr-salt chopped (all p<0.001). The 12hr-salt chopped almonds were also statistically lower than all the whole treated and untreated almonds, whereas it was significantly higher than all

the chopped soaked treatments (all p≤0.010). No statistically significant difference was observed between 12hr and 4hr whole almonds soaked in salt solution, but the potassium content of the whole almonds soaked for 12hr with salt was significantly lower than the whole almonds soaked for 12hr without salt (p=0.020). Furthermore, the potassium content was significantly lower for all chopped nuts compared to all whole nut treatments (all p<0.001).

5.3.6 Sodium

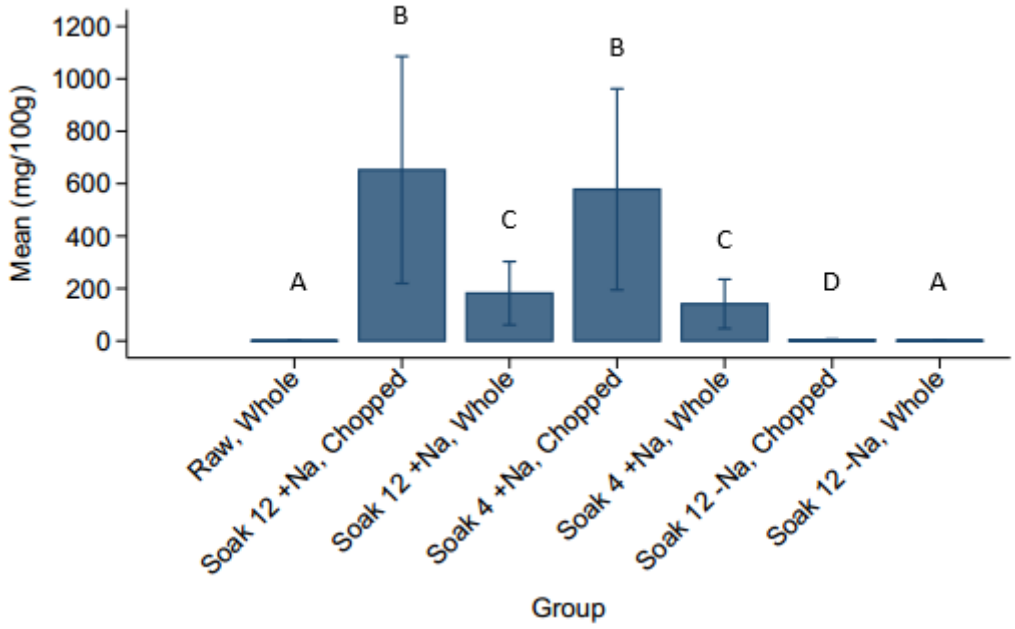

Figure 9: The mean sodium content for untreated and treated almonds.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The mean sodium concentration for untreated almonds was <0.8 mg/100g and the sodium in the treated almonds ranged from 1.5 to 653 mg/100g with the highest sodium in chopped almonds soaked for 12 hours in salt solution **(Table 6)**. All the treated almonds were statistically significantly different to untreated almond (p≤0.001), apart from 12hr-salt whole (p=0.191) **(Figure 9)**. There was a statistically significant

difference between whole and chopped for all soaking treatments, with the sodium content consistently higher among the chopped almonds (all p≤0.044). There was no statistically significant difference between the 4 and 12 hour soaking lengths for whole almonds (p=0.597) or chopped almonds soaked in salt solutions (p=0.801). Furthermore, the sodium content of 12hr+salt chopped almonds was significantly higher compared to 12hr-salt whole treatment. In addition, 12hr-salt chopped had a higher salt content compared to 12hr-salt whole and untreated (p≤0.044).

5.3.7 Zinc

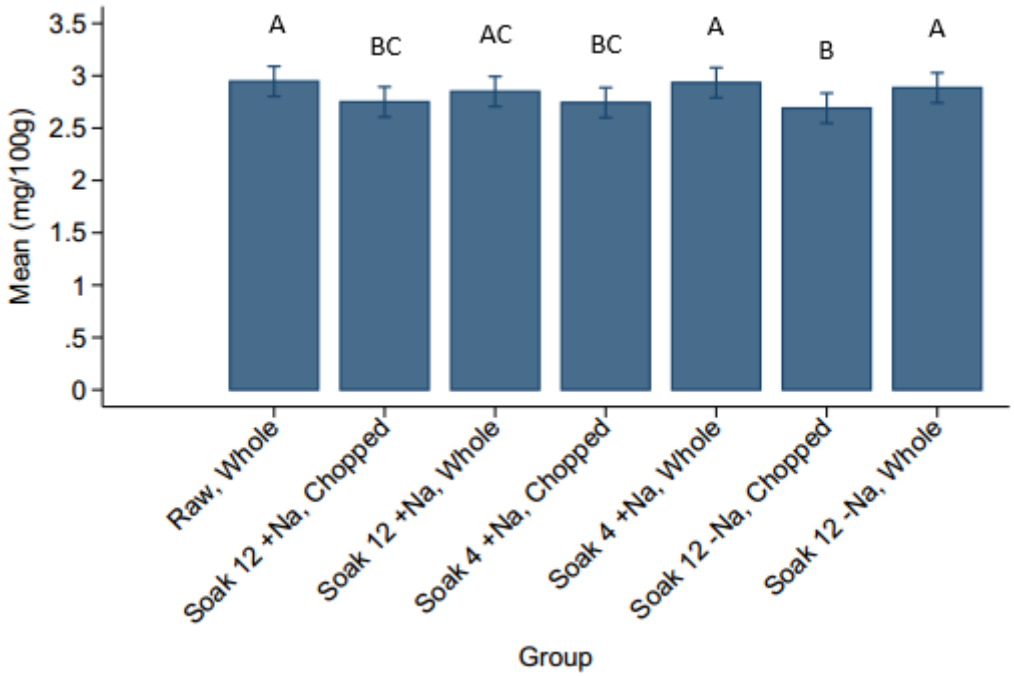

Figure 10: The mean zinc content for untreated and treated almonds.
Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The zinc concentration of untreated almonds was 2.9 mg/100g. In the treated almonds zinc concentrations ranged from 2.7-2.9 mg/100g, where zinc was higher in whole almonds than in chopped almonds **(Table 6)**. There was evidence of a difference in zinc

content between treatments (overall $p<0.001$) **(Figure 10)**. When untreated almonds were compared to all whole treated almonds no statistically significant differences were observed. Whereas chopped almonds showed a statistically significant decrease in zinc content compared to the untreated almonds (all $p\leq0.001$). Overall, all the chopped almond treatments had a significantly lower zinc content compared to the whole almonds, except for 12hr+salt chopped and 4hr+salt chopped which were not different to 12hr+salt whole. When considering each soaking treatment, there were statistically significant differences between whole and chopped where the latter was significantly lower (both $p=0.001$), except for 12+salt treatment ($p=0.094$). However, no statistically significant differences were observed when comparing all the whole almond treatments ($p\geq0.154$) and all the chopped almonds treatments ($p\geq0.311$).

Table 7: Mean (95% CI) phytate and mineral content of Hazelnuts for the different treatments[1]

Nutrient	Untreated	Soaked 12h+salt, chopped	Soaked 12h+salt, whole	Soaked 4h+salt, chopped	Soaked 4h+salt, whole	Soaked 12h-salt, chopped	Soaked12h-salt, whole	Overall p-value
Phytate (mg/100g)	482 (455, 509) ^{A}	411 (384, 438) ^{B}	466 (439, 493) ^{AC}	439 (412, 466) ^{BC}	477 (450, 504) ^{A}	414 (387, 441) ^{B}	464 (437, 491) ^{AC}	**<0.001**
Calcium (mg/100g)	147 (139, 155) ^{A}	128 (120, 136) ^{B}	139 (131, 147) ^{CD}	127 (119, 135) ^{B}	140 (132, 147) ^{ACD}	131 (124, 139) ^{BC}	144 (136, 152) ^{AD}	**<0.001**
Iron (mg/100g)	3.1 (2.8, 3.3) ^{A}	2.6 (2.5, 2.8) ^{B}	2.9 (2.7, 3.1) ^{AC}	2.7 (2.5, 2.9) ^{BD}	3.1 (2.9, 3.3) ^{A}	2.7 (2.5, 2.9) ^{BD}	2.9 (2.7, 3.1) ^{CD}	**<0.001**
Magnesium (mg/100g)	144 (132, 156) ^{A}	108 (99, 117) ^{B}	137 (126, 149) ^{AC}	128 (118, 139) ^{CD}	138 (127, 150) ^{AC}	121 (111, 131) ^{D}	140 (128, 152) ^{AC}	**<0.001**
Phosphorous (mg/100g)	295 (268, 321) ^{A}	227 (207, 248) ^{B}	288 (262, 314) ^{A}	274 (249, 298) ^{A}	290 (264, 316) ^{A}	230 (209, 250) ^{B}	284 (258, 309) ^{A}	**<0.001**
Potassium (mg/100g)	666 (609, 724) ^{A}	271 (213, 329) ^{B}	515 (457, 573) ^{C}	374 (316, 432) ^{D}	575 (517, 633) ^{E}	375 (317, 433) ^{D}	591 (533, 649) ^{E}	**<0.001**
Sodium (mg/100g)	0.8 (0.4, 1.2) ^{A}	489 (249, 730) ^{B}	219 (111, 327) ^{C}	501 (255, 748) ^{B}	169 (86, 253) ^{C}	0.9 (0.4, 1.3) ^{A}	2.4 (1.2, 3.5) ^{D}	**<0.001**
Zinc (mg/100g)	2.1 (2.0, 2.2) ^{A}	1.9 (1.8, 2.1) ^{A}	2.0 (1.8, 2.1) ^{A}	2.0 (1.9, 2.1) ^{A}	2.0 (1.9, 2.1) ^{A}	1.9 (1.8, 2.0) ^{A}	2.0 (1.9, 2.1) ^{A}	**0.581**

[1] Values with different superscript letters are statistically significantly different P<0.05

5.4 Phytate content in hazelnuts

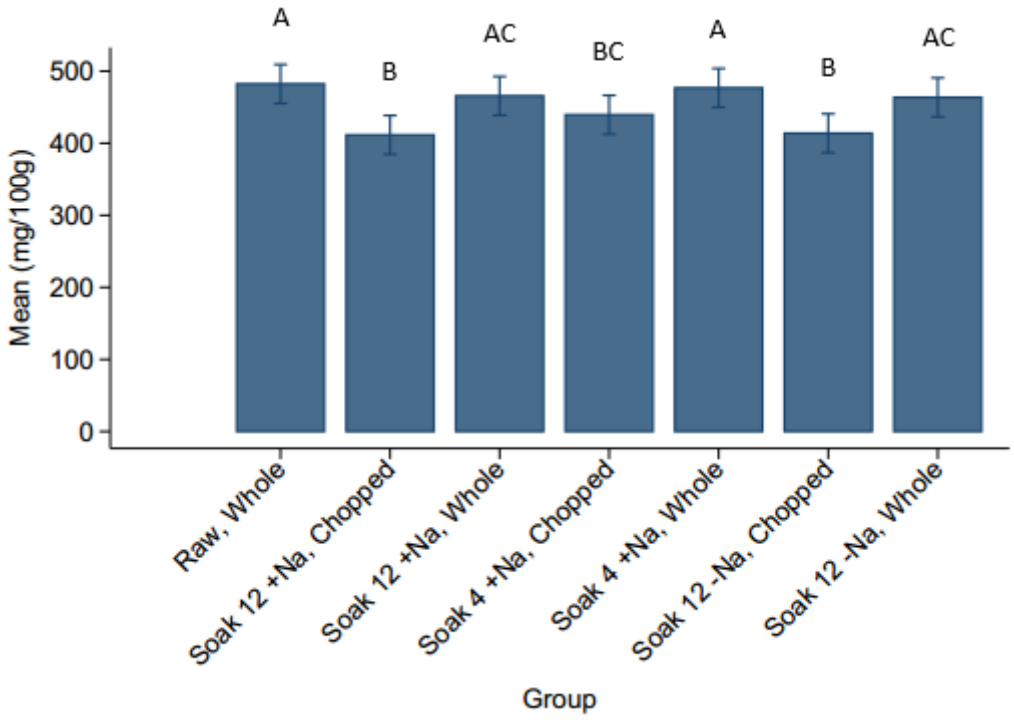

Figure 11: The mean phytate content for untreated and treated hazelnuts.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The untreated hazelnuts had a mean (95% CI) phytate content of 482 mg/100g (455, 509), with the phytate content of the treated hazelnuts ranging from 411 to 477 mg/100g **(Table 7)**. An overall statistically significant difference between hazelnut treatments was observed (p<0.001) **(Figure 11)**. Compared to the untreated hazelnuts, all the chopped hazelnut treatments were significantly lower (p≤0.004), whereas there was no difference for whole hazelnuts. Furthermore, no statistically significant differences were observed across all whole treated hazelnuts (p≥0.215). The hazelnuts which were chopped and soaked for 12 hours with or without salt had the lowest phytate content although there was no statistically significant difference across any soaking treatments with chopped hazelnuts. All the chopped hazelnut values were statistically significantly

lower compared to all whole treated hazelnut (all p≤0.001) except for 4hr+salt chopped which was only significantly lower to its corresponding whole hazelnuts (p=0.013).

5.5 Mineral content in hazelnuts

5.5.1 Calcium

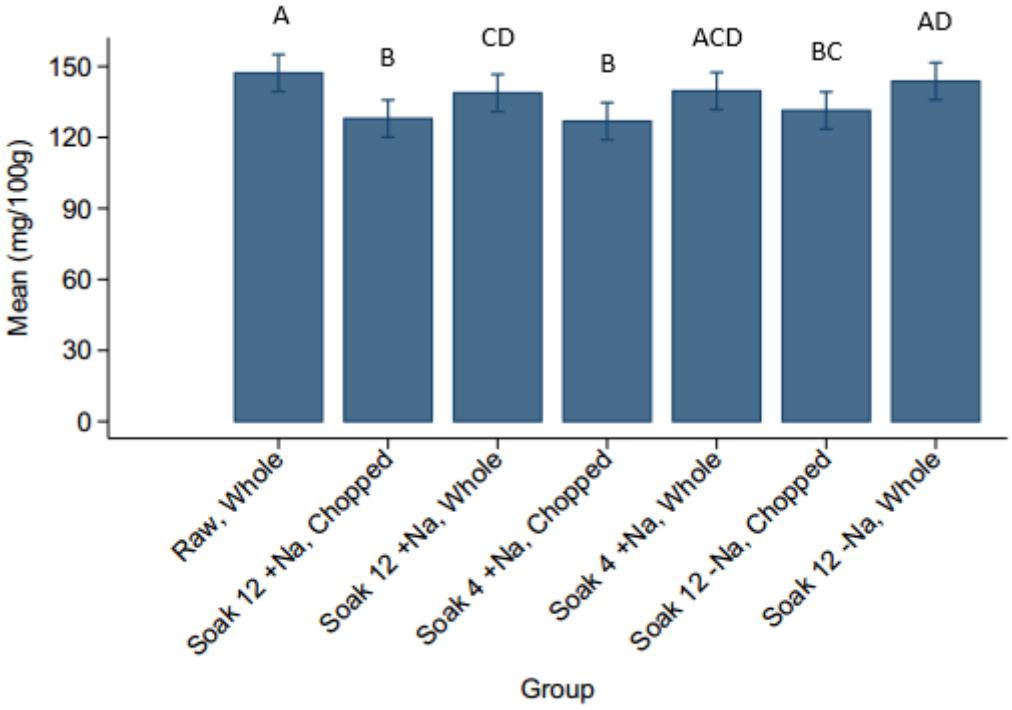

Figure 12: The mean calcium content for untreated and treated hazelnuts.
Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The calcium content for untreated hazelnuts (147 mg/100g) was statistically significantly different to all the treated hazelnuts ranging from 127-144 mg/100g (all p≤0.050) except for 4hr+salt whole and 12hr-salt whole **(Table 7) (Figure 12)**. Additionally, all the soaking treatments had a statistically significant difference between whole and chopped hazelnut where calcium was lower in chopped hazelnuts (p≤0.011). Although, no difference was observed across all whole treated hazelnuts (p≥0.249) and across all chopped treated hazelnuts (p≥0.426).

5.5.2 Iron

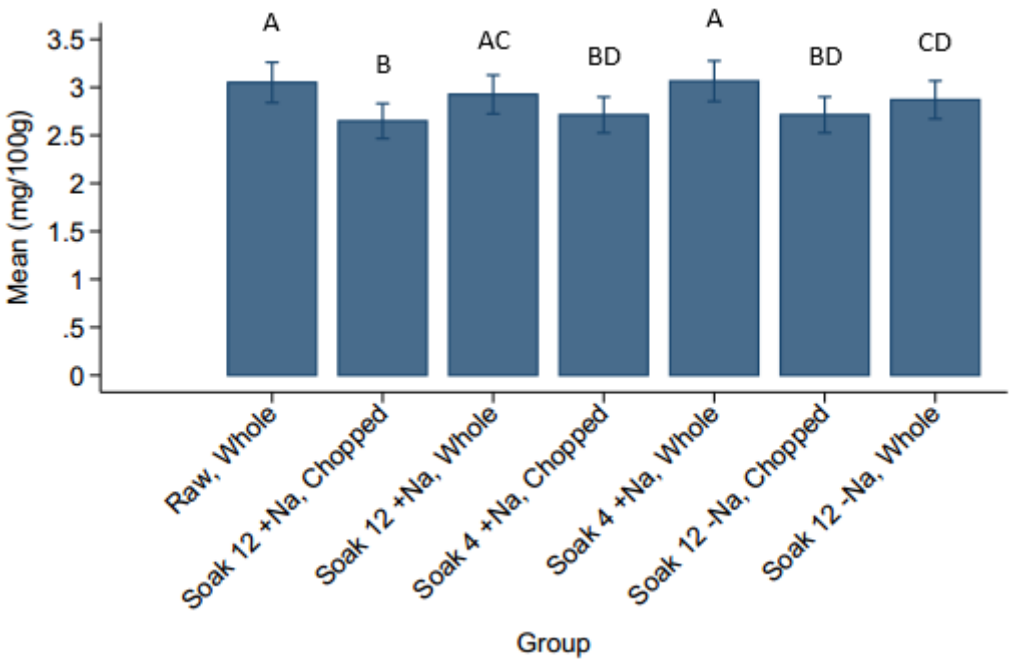

Figure 13: The mean iron content for untreated and treated hazelnuts.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The iron content in hazelnuts was 3.1 mg/100g in untreated whereas in whole treated hazelnut it ranged from 2.9-3.1 mg/100g, and 2.6-2.7 mg/100g in chopped treated hazelnut **(Table 7)**. All treated hazelnuts had a statistically significantly lower iron content than untreated hazelnuts (all $p \leq 0.048$) except for 12hr+salt whole and 4hr+salt whole **(Figure 13)**. There was a statistically significant difference between whole and chopped for soaking treatments 12hr+salt and 4hr+salt (lower in the chopped treatments) but not 12hr-salt. Further analysis showed no statistically significant differences between all the chopped treated hazelnuts. Although for whole treated, 12hr-salt whole was statistically significantly different to 4hr+salt whole ($p=0.033$) but not 12hr+salt whole ($p=0.531$).

5.5.3 Magnesium

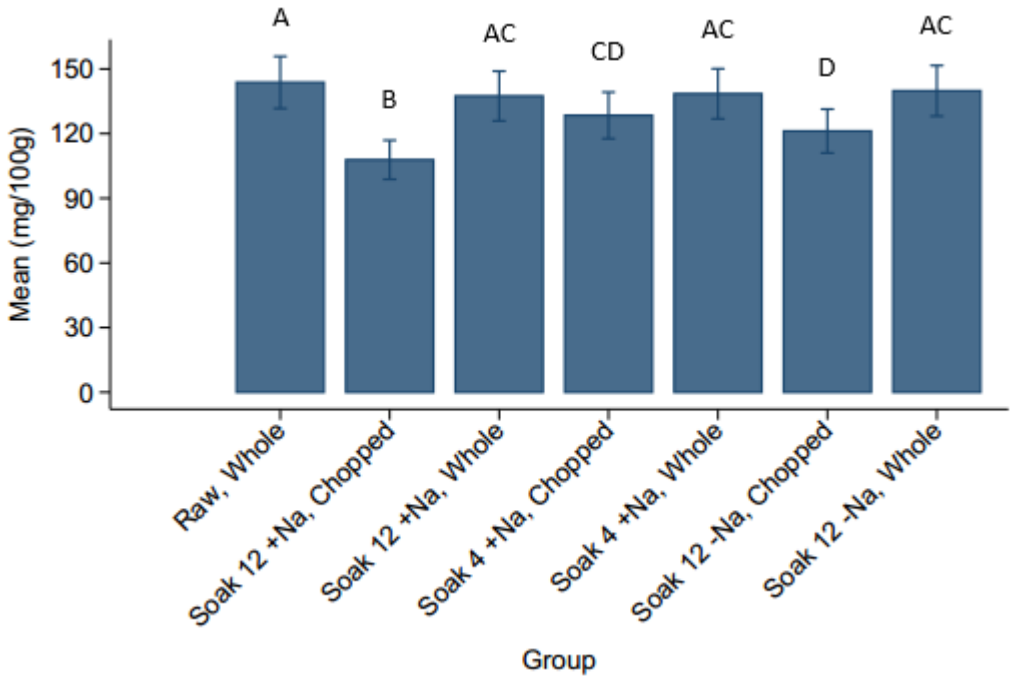

Figure 14: The mean magnesium content for untreated and treated hazelnuts.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The magnesium concentration for untreated hazelnut (144 mg/100g) was statistically significantly higher compared to all the chopped treated hazelnuts (108-128 mg/100g) (all p≤0.014), however, no differences were observed when comparison was made with all the whole treated hazelnuts (137-140 mg/100g) (p≥0.327) **(Table 7) (Figure 14)**. There were significant differences in treatment groups between whole and chopped except for 4hr+salt chopped where there was no evidence of a statistical difference with any of the whole treated hazelnuts. Additionally, there were no differences across all whole treated hazelnuts (≥0.706), although chopped treated hazelnuts were statistically significantly different to each other (all p≤0.011), except for 4hr+salt chopped and 12hr-salt chopped (p=0.203).

5.5.4 Phosphorus

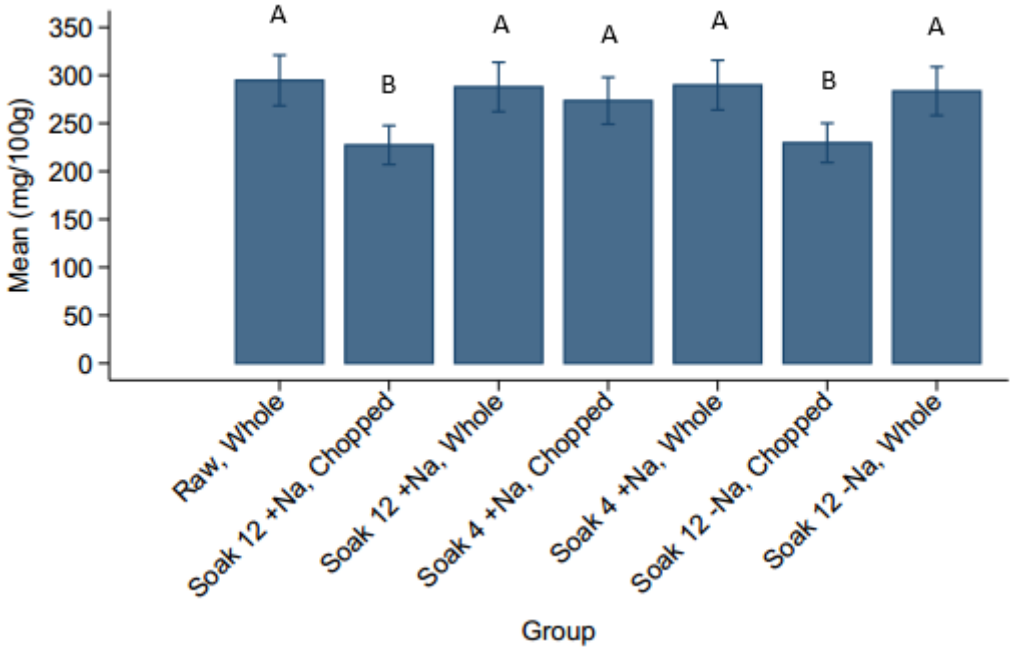

Figure 15: The mean phosphorus content for untreated and treated hazelnuts.
Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The mean phosphorus content in hazelnuts was 295 mg/100g, whereas phosphorus in whole hazelnuts ranged from 284-290 mg/100g and 227-274 mg/100g in chopped hazelnuts **(Table 7)**. No statistical difference was observed between whole treated hazelnuts and untreated hazelnuts **(Figure 15)**. However, all chopped hazelnuts except for 4hr+salt were significantly lower compared to untreated and all whole treated hazelnuts (all $p<0.001$). Furthermore, no statistically significant differences were seen between all whole treated hazelnuts, however, 4hr+salt chopped hazelnuts was statistically significantly different to 12+salt and 12hr-salt chopped hazelnuts.

5.5.5 Potassium

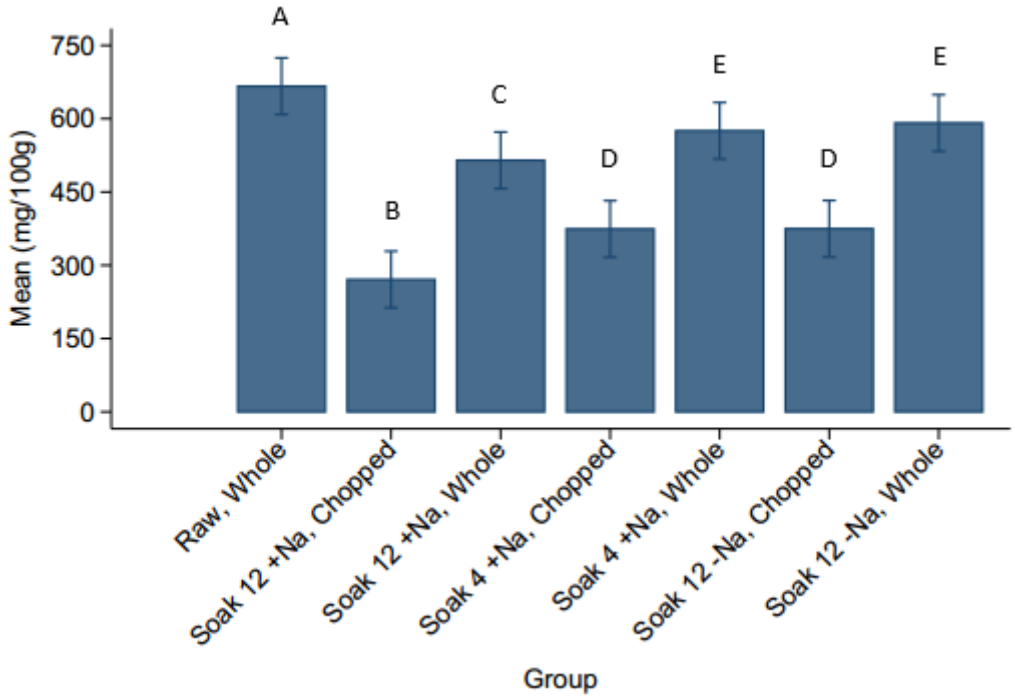

Figure 16: The mean potassium content for untreated and treated hazelnuts.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The untreated hazelnuts had a mean potassium content of 666 mg/100g; the potassium in treated whole hazelnuts ranged from 515-591 mg/100g and 271-375 mg/100g for treated chopped hazelnuts **(Table 7)**. All treated hazelnuts had a significantly lower potassium content compared to untreated hazelnuts (all p≤0.008) **(Figure 16)**. The highest reduction was observed for 12hr+salt chopped, which was also statistically significantly different to 4hr+salt chopped and 12hr-salt chopped. Furthermore, 12hr+salt chopped and 12hr+salt whole had significantly lower potassium content than all other chopped and whole hazelnuts when comparison was made with other soaking treatments. A statistically significant difference was also evident for all soaking treatments between whole and chopped (lower in chopped treatments) (all p<0.001). However, 4hr+salt chopped was not statistically significantly different to 12hr-salt

chopped and 4hr+salt whole and 12hr-salt whole did not differ significantly (all p≥0.573).

5.5.6 Sodium

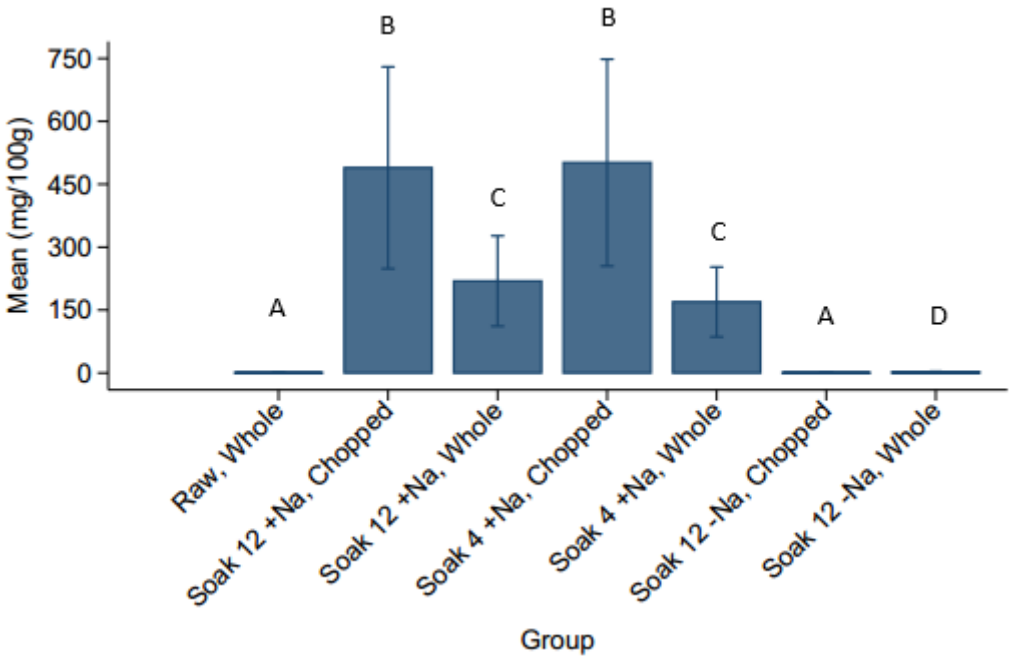

Figure 17: The mean sodium content for untreated and treated hazelnuts.
Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The mean sodium concentration for untreated hazelnut was <0.8 mg/100g and the sodium content in treated hazelnuts ranged from 0.9 to 501 mg/100g with the highest sodium in chopped hazelnuts soaked for 4 hours in salt solution **(Table 7)**. All the treated hazelnuts were statistically significantly higher than untreated hazelnuts (all p≤0.002), apart from 12-salt chopped (p=0.815) **(Figure 17)**. For each treatment, chopping the hazelnuts had a significant effect on sodium content, with the chopped hazelnuts having a statistically significantly higher sodium content than the whole hazelnuts. No statistically significant differences were observed between the two whole hazelnuts, and between the two chopped hazelnuts soaked in salt solutions for 12hrs and

4 hours (all p≥0.457). Furthermore, 12hr-salt whole had a significantly higher sodium content compared to 12hr-salt chopped and untreated hazelnuts (all p≤0.004).

5.5.7 Zinc

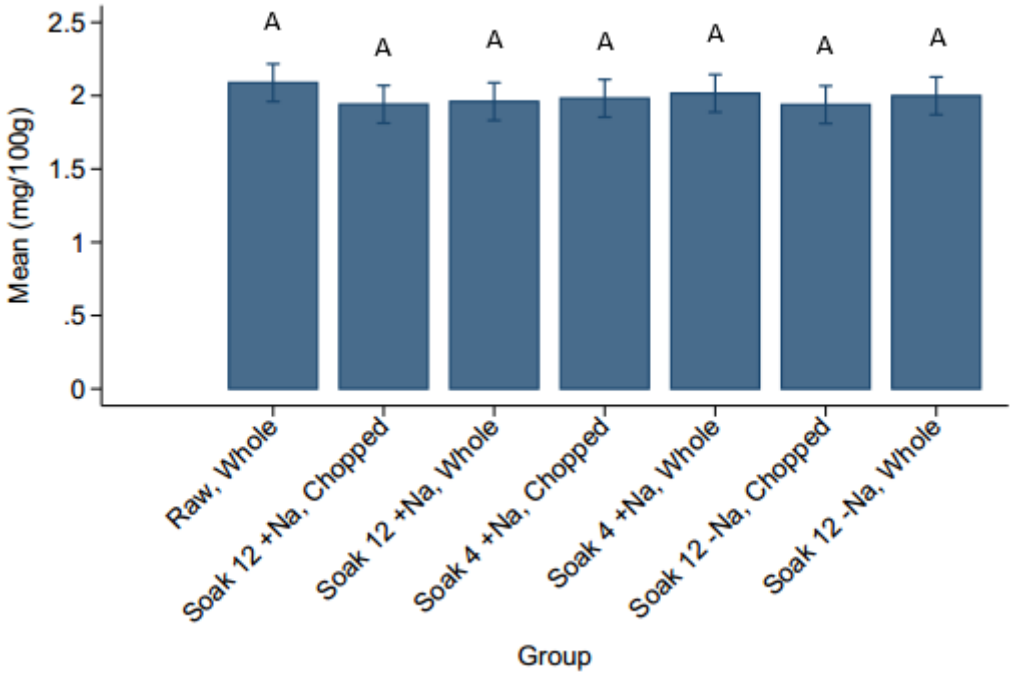

Figure 18: The mean zinc content for untreated and treated hazelnuts.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

The mean zinc content in hazelnuts was 2.1 mg/100g whereas the treated hazelnuts ranged from 1.9-2.0 mg/100g **(Table 7)**. There was no evidence of an overall statistically significant difference between treatments (overall p=0.581) **(Figure 18)**. Therefore, chopping hazelnuts, different soaking durations and addition of salt to the soaking solution had no statistically significant effects on zinc concentrations in hazelnuts.

Table 8: The mean molar ratios of phytate: zinc, phytate: iron, phytate: calcium and phytate x calcium: zinc for Almond and hazelnuts[1]

	Untreated	Soaked 12hr+ salt, chopped	Soaked 12hr+ salt, whole	Soaked 4hr+salt, chopped	Soaked 4hr+salt, whole	Soaked 12hr-salt, chopped	Soaked 12hr-salt, whole	Overall p-value
Almond								
Phytate: Zinc ratio	17.6 ^{A}	18.3 ^{AB}	19.1 ^{AB}	19.5 ^{B}	19.3 ^{B}	19.0 ^{AB}	19.2 ^{AB}	**0.205**
Phytate: Iron ratio	12.9 ^{A}	14.4 ^{BC}	13.8 ^{AB}	15.2 ^{C}	13.9 ^{AB}	14.8 ^{BC}	14.2 ^{BC}	**0.002**
Phytate: Calcium	0.13 ^{A}	0.13 ^{AB}	0.14 ^{B}	0.14 ^{B}	0.14 ^{B}	0.13 ^{AB}	0.13 ^{AB}	**0.059**
Phytate x Calcium: Zinc ratio	113 ^{AB}	106 ^{A}	113 ^{AB}	113 ^{AB}	117 ^{BC}	112 ^{AB}	123 ^{C}	**0.034**
Hazelnut								
Phytate: Zinc ratio	23.1 ^{AB}	21.0 ^{A}	23.6 ^{B}	22.1 ^{AB}	23.5 ^{B}	21.1 ^{A}	23.0 ^{AB}	**0.108**
Phytate: Iron ratio	13.4 ^{A}	13.1 ^{A}	13.5 ^{A}	13.8 ^{A}	13.1 ^{A}	12.8 ^{A}	13.7 ^{A}	**0.778**
Phytate: Calcium	0.20 ^{AB}	0.19 ^{AB}	0.20 ^{AB}	0.21 ^{B}	0.21 ^{AB}	0.19 ^{A}	0.20 ^{AB}	**0.307**
Phytate x Calcium: Zinc ratio	84 ^{A}	67 ^{B}	81 ^{A}	70 ^{B}	82 ^{A}	69 ^{B}	82 ^{A}	**<0.001**

[1] Values with different superscript letters are statistically significantly different P<0.05

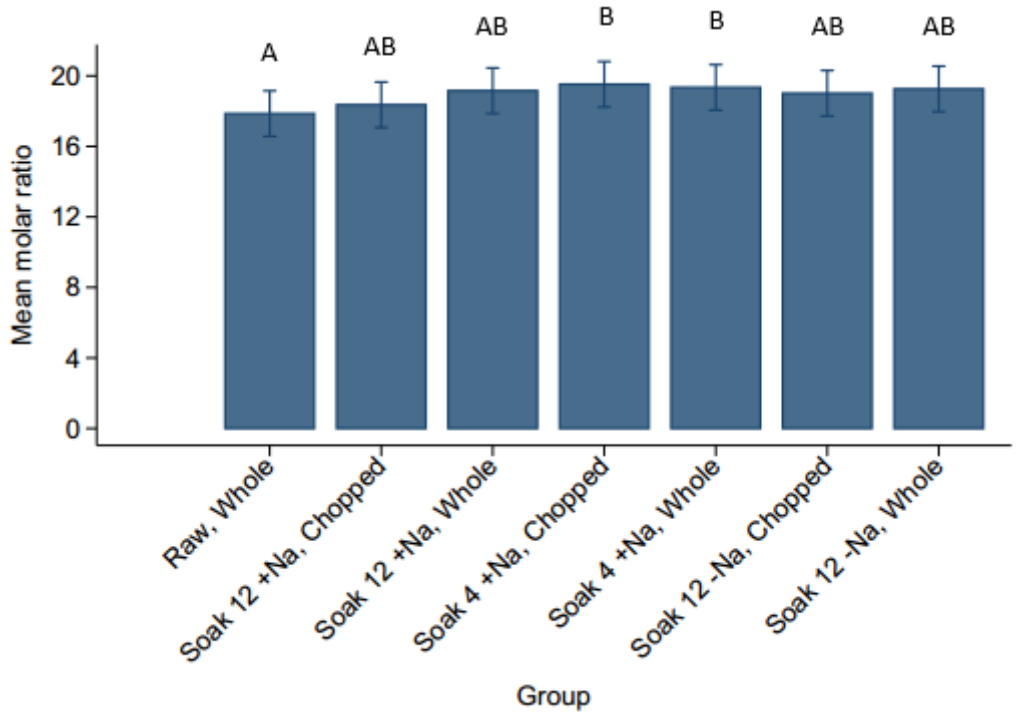

Figure 19: The mean phytate to zinc molar ratio for untreated and treated almonds

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

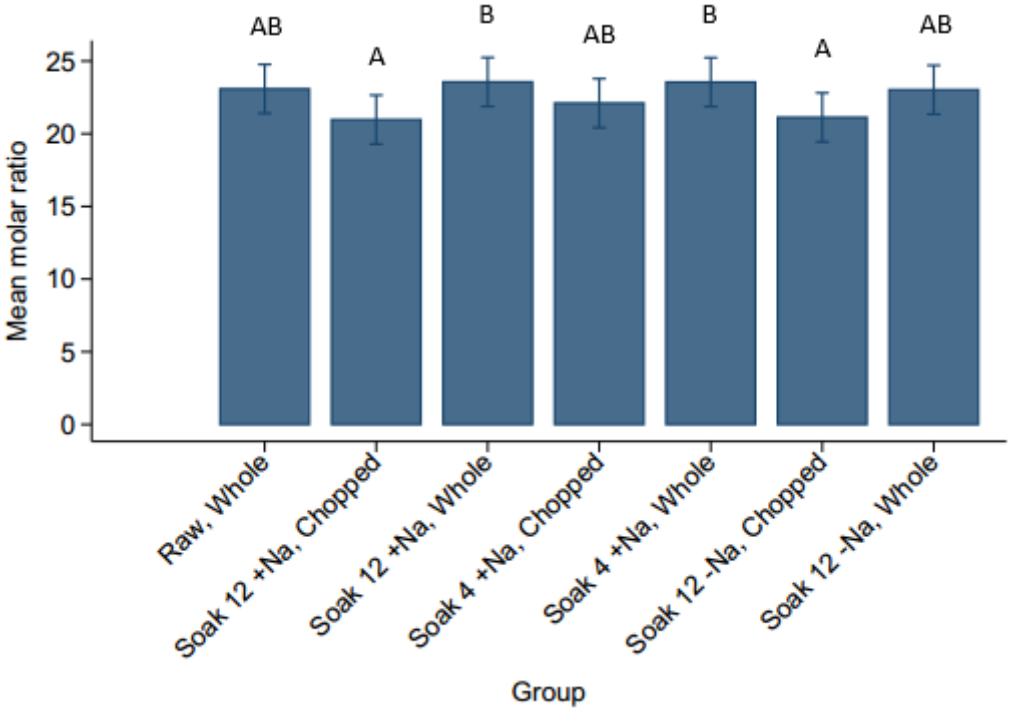

Figure 20: The mean phytate to zinc molar ratio for treated and untreated hazelnuts

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

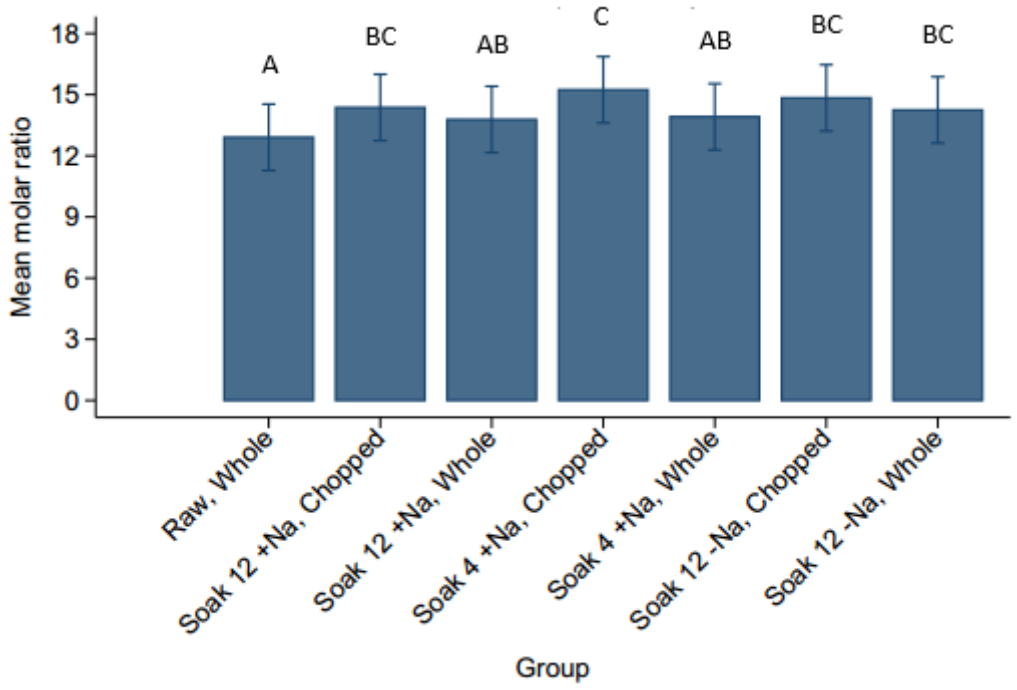

Figure 21: The mean phytate to iron molar ratio for treated and untreated almond

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

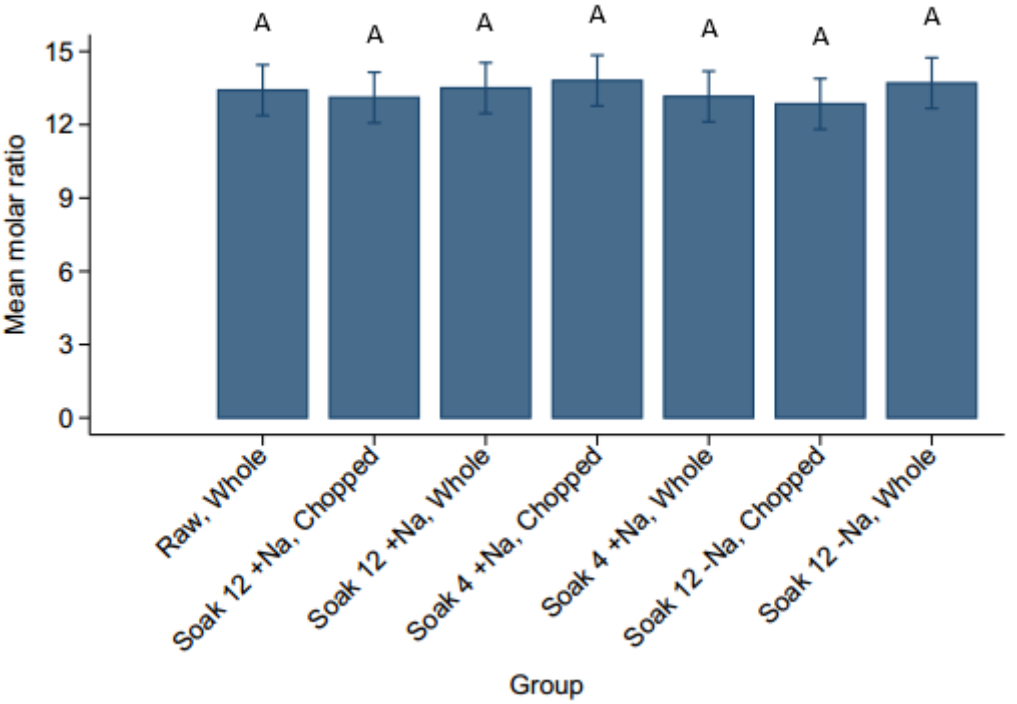

Figure 22: The mean phytate to iron molar ratio for treated and untreated hazelnuts

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

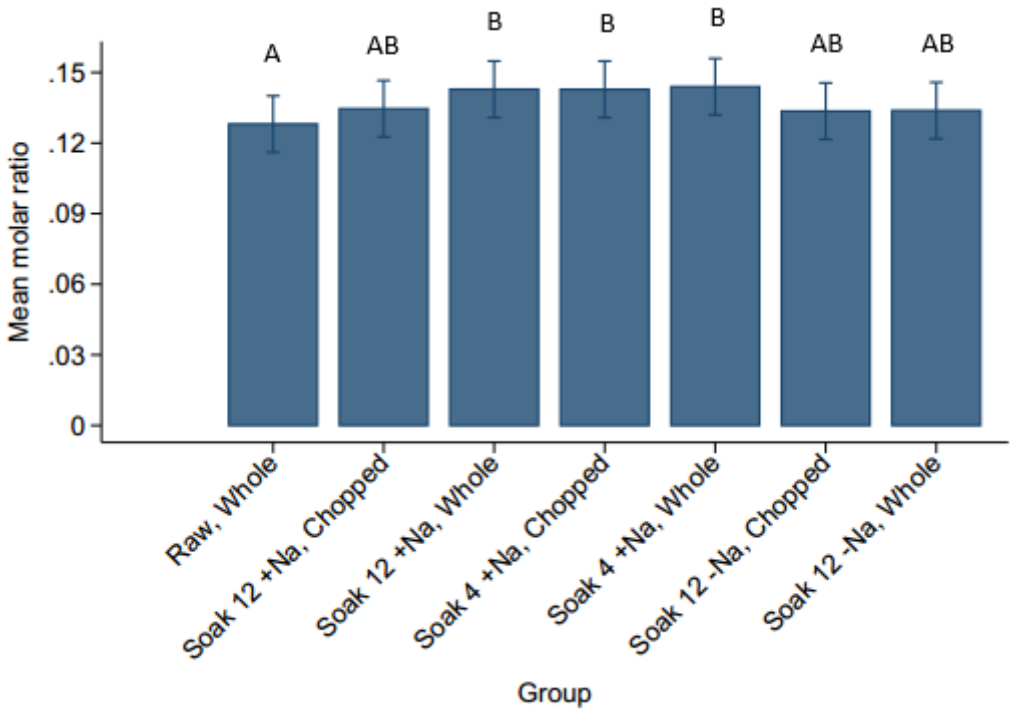

Figure 23: The mean phytate to calcium molar ratio for treated and untreated almond.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

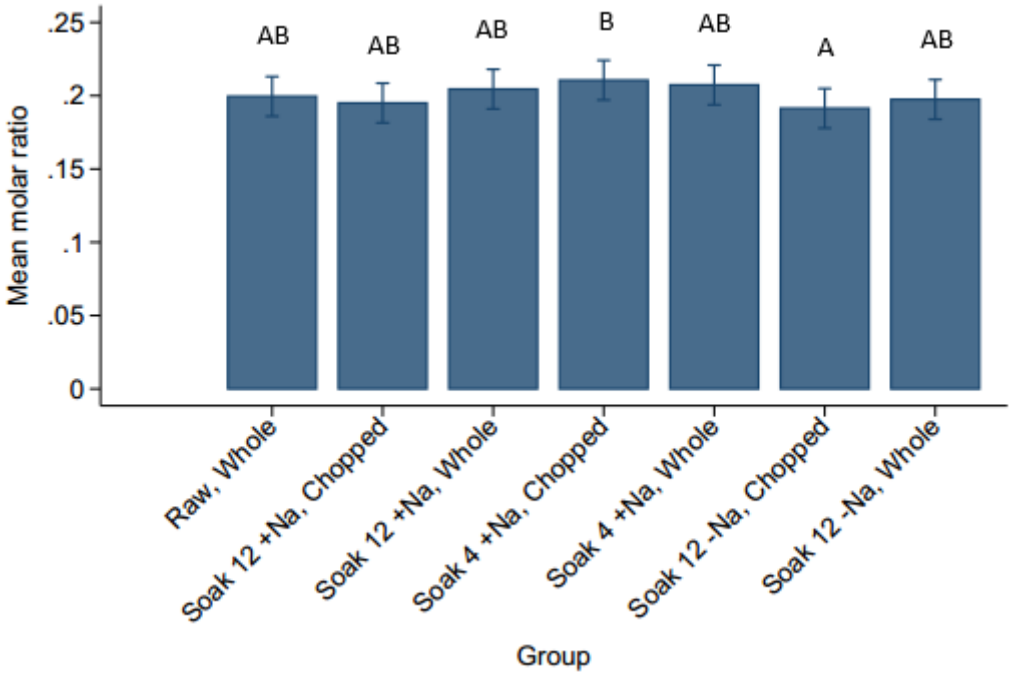

Figure 24: The mean phytate to calcium molar ratio for treated and untreated hazelnuts.

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

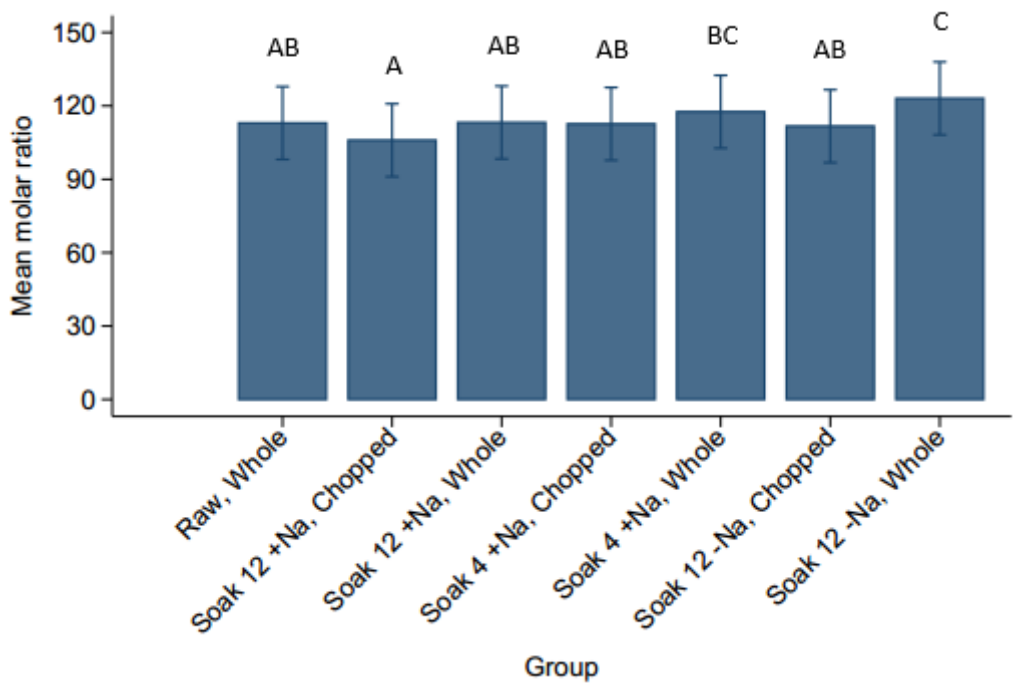

Figure 25: The mean phytate x calcium to zinc molar ratio for treated and untreated almond

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

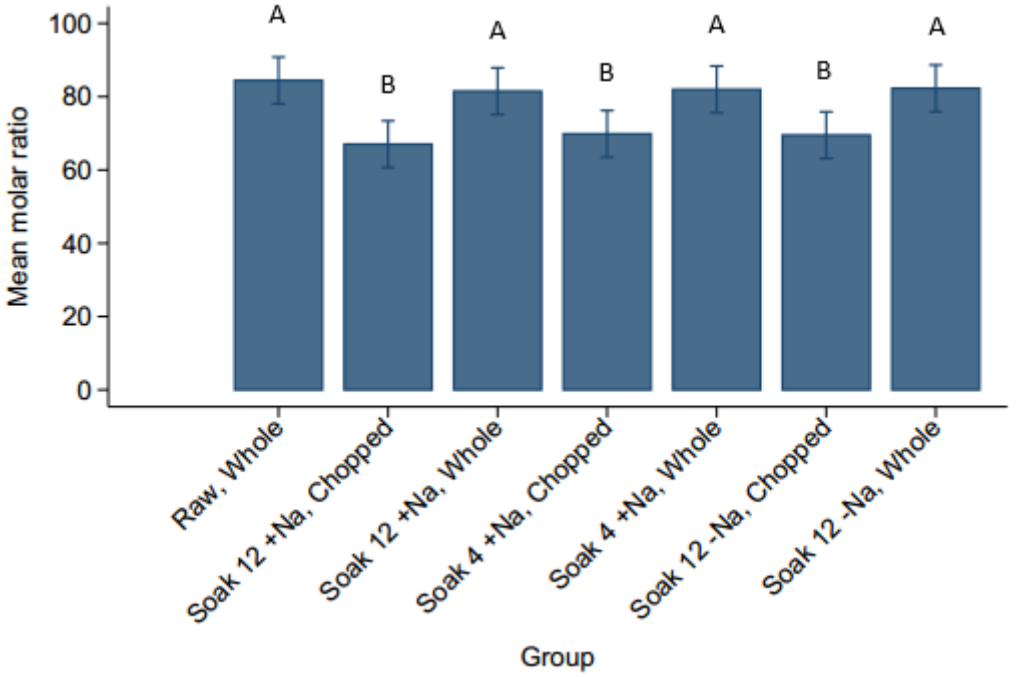

Figure 26: The mean phytate x calcium to zinc molar ratio for treated and untreated hazelnuts

Values are expressed as mean ± 95% CI; values with different superscript letters are statistically significantly different P<0.05.

5.6 The molar ratios of phytate: zinc, phytate: iron, phytate: calcium, and phytate x calcium: zinc for almonds and hazelnuts

Table 8 and Figures 19-26 presents the molar ratios of phytate to zinc, iron, calcium and phytate x calcium:zinc for both almonds and hazelnuts. The mean ratios of phytate to zinc, iron, calcium and phytate x calcium:zinc for untreated almonds were 17.6, 12.9, 0.13 and 113, respectively. There was no evidence of an overall significant difference between treatments for phytate:zinc and phytate:calcium. However, there was evidence of an overall significant difference between treatment arms for the phytate:iron molar ratio (p=0.002) and phytate x calcium:zinc (p=0.034). Pairwise comparisons showed that the phytate:iron molar ratio was statistically significantly higher for 12hr+salt chopped, 4hr+salt chopped, 12hr-salt chopped and 12hr-salt whole compared to untreated almonds, although no difference was observed between the four treatment groups. Additionally, there was a statistically significant difference in the treatment arm 4hr+salt between chopped and whole (p=0.021), however, no statistically significant differences were observed when comparing between all whole treatments and all chopped almond treatments. Pairwise comparison for phytate x calcium:zinc molar ratio showed that 12hr-salt whole was the only treatment group that was statistically significantly higher compared to untreated almonds (p=0.015). Furthermore, 12hr-salt whole had a significantly higher phytate x calcium:zinc molar ratio than 12hr+salt whole (p=0.018), also, there was a statistically significant difference between 12hr-salt chopped and 12hr-salt whole, where latter was significantly higher (p=0.006).

The mean ratio of phytate to zinc, iron, calcium and phytate x calcium:zinc for untreated hazelnuts were 23.1, 13.4, 0.20 and 84, respectively. An overall significant difference

was not evident for hazelnuts for the phytate:zinc, phytate:iron and phytate:calcium molar ratios. However, there was evidence of an overall significant difference in the phytate x calcium/zinc ratio between the treatment groups ($p<0.001$). All the chopped treated hazelnuts had a significantly lower molar ratio for phytate x calcium:zinc compared to untreated hazelnuts (all $p<0.001$). However, there were no statistically significant differences between whole treated hazelnuts and the untreated hazelnuts. In addition, all of the chopped treatments had ratios statistically significantly lower compared to the whole hazelnut treatments (all $p\leq0.003$).

6 Discussion

6.1 Results Summary

To the best of my knowledge, this is the first study to assess the effects of soaking different forms of almonds and hazelnuts on both phytate and mineral concentrations. The current study found no evidence that any form of soaking was effective in reducing phytate concentration in whole almonds or hazelnuts, although most soaking treatments showed reductions in potassium in the whole forms, but inconsistent reductions were found for calcium. However, for chopped nuts, the soaking process resulted in statistically significant decreases in phytate concentrations, although the majority of the minerals (calcium, iron, magnesium, phosphorus, potassium, and zinc) were also statistically significantly reduced by the soaking process, aside from zinc for most treatments of chopped hazelnuts. Analysis of the phytate:mineral ratios suggest that soaking nuts does not result in clinically meaningful improvements in the bioavailability of zinc, calcium and iron. In contrast, sodium content substantially increased for all nuts following soaking in salt solutions. Furthermore, in some cases the addition of salt was found to influence the reduction of calcium, potassium and magnesium in the nuts.

6.2 Effect of soaking almonds and hazelnuts on phytate content

In the current study, soaking was not effective in reducing phytate content in whole almonds or hazelnuts. Surprisingly, a statistically significant increase in phytate content of 7.6% was observed after soaking whole almonds for four hours with added salt. This finding was unexpected and may be a chance result given the number of significance tests performed. However, soaking was generally effective in reducing phytate in chopped hazelnuts with reductions around 10%. It is likely that the reductions in phytate

observed were predominantly the result of passive diffusion/leaching (26, 76). Chopping the nuts increased the surface area and exposed the inner layers of the nuts (possibly where phytate is located) hence allowing more phytate molecules to leach out into the soaking solution. Another possible mechanism for phytate reduction could be through hydrolysis of phytate using the endogenous enzyme phytase, but this was not assessed in the current study (16).

To date only one study has examined the effects of soaking on the phytate content of almonds (75). That study is not entirely comparable to the current research as the almonds were soaked for longer with a lower drying temperature. They reported an increase in phytic acid content when whole almonds were soaked in water for 15hr at 25°C, which increased when soaked at 40°C (75). No explanation for the increase in phytate was provided. It is important to note the authors did not adjust the phytate results for possible moisture loss during drying. Furthermore, the authors used an indirect method of phytate analysis, where all the phosphorus in the almonds was assumed to be from phytate, and therefore overestimation of phytic acid content is likely.

In contrast, studies that assessed the effects of soaking grains and legumes are consistent with the findings of the current study. In general, grains and legumes that were soaked after reducing the particle size had greater reductions in phytate than their whole counterparts (24). For example, Kruger et al. reported soaking milled sorghum and maize resulted in greater phytate reduction (39% and 57%, respectively) compared to unmilled sorghum and maize (13% and 14%, respectively) (29). Similar results were seen by Hotz et al. where a 57% and 51% reduction in phytate was seen after soaking milled and pounded maize, respectively (76). Collectively, these results suggest the

degree of milling affects the amount of phytate reductions. Although, the degree of phytate reduction in these studies on grains and legumes appear higher than what was achieved here in nuts. This difference between nuts and grains and legumes is possibly due to structural and biochemical differences.

6.3 Effect of soaking almonds and hazelnuts on mineral content

Generally, soaking whole almonds and hazelnuts had little or no influence on iron, calcium, magnesium, phosphorus and zinc concentrations. Whereas, whole almonds and hazelnuts showed overall evidence for decreases in potassium with reductions of 5-13% and 11-23%, respectively. On the other hand, for chopped almonds and hazelnuts, soaking reduced mineral content to a greater degree than whole nuts: calcium (7-9% and 11-14%, respectively), iron (14% and 10-13%, respectively), magnesium (12-16% and 11-25%, respectively), phosphorus (8-10% and 7-23%, respectively although one of the three treatments for hazelnuts was not statistically significant), potassium (25-38% and 44-59%, respectively), and for almonds only, zinc (7%).

Potassium showed the greatest reduction upon soaking for both almonds and hazelnuts, particularly when chopped. This reduction could be due to potassium's ionic nature, which means it cannot covalently bond to the nut matrix, so it freely diffuses into the soaking solution much easier compared to the other elements analysed. Additionally, calcium content decreased in whole almonds and hazelnuts only when soaked for 12 hours in salt solution.

As expected the sodium content for both whole and chopped almonds (141-182 mg/100g and 578-653 mg/100g respectively) and hazelnuts (169-219 mg/100g and 489-501 mg/100g, respectively) increased significantly when soaked in a salt solution, with greater increases observed for the chopped form. When compared to the suggested

dietary target (SDT) for sodium (1600 mg/day), consuming 30 g of whole nut (55-66 mg) and chopped nut (150-196 mg), soaked nuts would contribute a further ~ 4%- 12% of the SDT (96). Given that population sodium intakes are usually higher than recommended, increasing the sodium content of a food such as nuts, which are naturally low in sodium, is undesirable (97). The increase in sodium content in chopped nuts was likely the result of increasing surface area of the nut allowing more sodium to move into the nut through passive diffusion.

One unanticipated finding was a lower degree of leaching of zinc compared to other minerals. This is probably due to the difference in the location of zinc in nuts and also the type of molecules the different minerals are attached to (30). Zinc has been found to have a structural role in numerous proteins and enzymes (30). This suggests that zinc bonding to these proteins shows covalent character and this type of bonding results in the slow diffusion of zinc from the nut matrix into the soaking solution (98).

Soaking duration and the addition of salt to the soaking solution generally had no statistically significant effects on calcium, iron, magnesium, phosphorus and zinc for whole and chopped almond, with a few exceptions. A reduction in calcium (chopped) and potassium (whole and chopped) content seemed to be influenced by the addition of salt to almonds. Similarly, sodium content in both chopped and whole almonds and hazelnuts were influenced only by the addition of salt to the soaking solution.

Increasing the length of soaking and addition of salt to the soaking solution both led to greater loss of magnesium (chopped) and potassium (whole and chopped) in hazelnuts. The mineral losses were further increased when both treatments were combined i.e. soaking 12hr+salt. In addition, the phosphorus content in chopped hazelnuts only decreased when the soaking duration was increased.

Overall, when chopped almonds and hazelnuts from all treatment groups were compared with their whole almond and hazelnut counter parts, the nutrient content for chopped nuts appeared to be significantly lower than whole nuts.

6.4 The phytate:mineral molar ratios for almonds and hazelnuts

Soaking almonds and hazelnuts had no statistically significant effect on phytate:zinc (above 18 and 21, respectively) and phytate:calcium (below 0.14 and 0.21, respectively) molar ratios, where ratios above 15 and 0.24, respectively are considered inhibitory (70). In fact, there was a non-statistically significant increase in phytate:zinc molar ratios after soaking almonds in all soaking treatments. A decrease in the phytate:zinc molar ratio was observed when hazelnuts were chopped but the magnitude of reduction in the ratio between soaking treatments was not substantial. The calcium molar ratios were inconsistent between the treatment groups for both almonds and hazelnuts. Even though these values were below 0.24 (ratio where bioavailability of calcium is not compromised), soaking was not influential in further increasing bioavailability of calcium.

The phytate:iron molar ratio increased in all the soaking treatments for almonds, ranging from 13-15, although, the molar ratios were higher among the chopped almonds compared to whole. This is because while there was greater phytate reduction in the chopped nuts, we also saw greater reductions in iron, resulting in a higher ratio. The molar ratios remained above the proposed critical level of 1, which suggests iron availability continued to be impaired despite soaking (63, 68-70). In contrast, the phytate:iron molar ratio in hazelnuts did not statistically significantly differ between treatments, and all ratios remained above 1.

It has been suggested that the phytate x calcium:zinc molar ratio is a better predictor of zinc bioavailability of some foods (68). It is purported a ratio above 200 compromises zinc bioavailability. None of the samples had ratios above this cut off. With soaking, the phytate x calcium:zinc molar ratios for almonds was either reduced or stayed the same for all but one soaking treatment. In contrast, the phytate x calcium:zinc molar ratios for hazelnuts decreased from 84 to 67, with the greatest reductions observed for chopped treatments. The higher molar ratios in almonds are probably due to the increase in phytate and higher calcium content, while in hazelnuts the phytate and calcium content was lower despite the fact that zinc content decreased similarly in both nuts.

No study to date has assessed both phytate and mineral bioavailability in nuts. Findings from studies involving cereals and legumes are somewhat inconsistent with the current study. Lestienne et al. reported no improvement for phytate:iron ratio and a slight decrease in phytate:zinc ratio after soaking whole cereals and legumes (30). In contrast, Afify et al. reported an increase in the phytate:iron ratio and a decrease in phytate:zinc ratio in sorghum after soaking (25). Conversely, Perlas et al. reported a substantial reduction in the phytate:iron ratio from 22 to 0.4 for rice flour after 12 hours of soaking (33).

Collectively, the results suggest that soaking whole nuts does not appear to affect minerals bioavailability. Although chopping nuts resulted in greater reductions in phytate concentrations, on the whole this did not improve mineral bioavailability because either the magnitude of phytate reduction was not sufficient, or it was accompanied by a reduction in mineral content which attenuated a reduction in the phytate:mineral ratio.

6.5 Strengths and limitations

A strength of the current study is that it replicated popular nut activation methods from the lay literature. This therefore provides results that reflect actual soaking practices and so can be used to inform pragmatic evidence-based health messages regarding the effect of soaking nuts. In order to enhance the generalisability of the results, the study nut samples consisted of five different brands of each nut type that were readily available in supermarkets (produced in New Zealand and other countries). This means that the results obtained in this study should be more generalisable to different botanical varieties, which may vary in terms of environmental conditions, locations, soil types, fertilizer application, year of production/harvest, different maturation stages, and storage (temperature and duration) (16, 50, 62, 65), than would be the case if only a single source or homogenised samples were used.

Our study has some limitations to bear in mind when interpreting the results. Firstly, it is important to note that the nuts which were soaked, were also dried for 24 hours at 65°C. It is possible that the changes in composition of soaked nuts did not solely reflect losses from soaking but was a combination of soaking and drying. Previous research in legumes have shown that heating may influence phytate content (65). However, the drying process used in the present study reflected the methods outlined in the lay literature, therefore, mimicking real-life as much as possible. Furthermore, the drying process appeared to reduce the water content of the soaked nuts compared to raw nuts, which were not exposed to drying. Therefore, weight adjusted phytate and mineral contents were calculated to account for the loss of water content. The weight adjustment also accounts for potential matter lost in the soaking process. This adjustment allowed for direct comparisons between raw untreated nuts and soaked nuts. If left unadjusted

the loss of weight observed with the soaking and drying process, which ranged from 1.7% to 12.8%, would have artificially concentrated the phytate and mineral content of the soaked nuts.

Furthermore, soaking time, volume of soaking liquid and removal of soaking liquid are all factors that could have potentially influenced the nutrient content of soaked nuts. Hotz and Gibson et al. found that longer soaking time, higher volume of soaking liquid and proper removal of soaking liquid increases the amount of phytate that is lost (76). Studies that used a lower grain:soaking solution ratio showed less phytate content reduction, although, changing the soaking solution showed a greater reduction (76, 77).

7 Conclusion and future research

It is clear from the current research that soaking almonds and hazelnuts in the whole form was not effective in reducing phytate, despite many claims in the lay literature suggesting that nuts should be 'activated' to enhance mineral bioavailability. While chopping the nuts led to greater reductions in phytate, the mineral content was also compromised, with no meaningful improvements observed in the phytate: mineral molar ratios. Furthermore, soaking duration and addition of salt to soaking solution was also not effective in phytate removal. Therefore, there is no evidence to support the claims that activating nuts reduces phytate content to the extent which allows for greater nutrient bioavailability.

While soaking nuts in the New Zealand context appears unnecessary, future research on soaking food products for vulnerable populations (e.g. vegetarian or vegan) could examine the effects of varying some of the parameters used in the current study and other literature, including changing: soaking water temperature, soaking environmental temperature, pH levels, rinsing after soaking as oppose to no rinsing, the water to nut ratio, increasing soaking duration to more than 12 hours, changing soaking water frequently (every 4 hours) and soaking nuts without the skin (pellicle) to further explore the area of studies in nuts. However, given the results of the current study, the magnitude of change obtained from these proposed studies seem unlikely to outweigh the negative aspect of soaking (decreased mineral content, time consuming and cost associated with drying and man power which are possible barriers to regular nut consumption). Therefore, a more fruitful area of research would be to explore public health strategies to improve nut consumption, given that nut consumption in New Zealand and other countries is inadequate in terms of both frequency and amount.

8 Application to Dietetic Practice

Nuts are known to be an important source of macronutrients (particularly cis-unsaturated fats), micronutrients and phytonutrients. Nut consumption as part of a healthy diet has been shown to reduce the risk of CVD and can also help with weight management (13). Currently the Ministry of Health dietary guidelines recommend 30g of nuts to be consumed daily to have maximal health benefits (12, 13). However, whole nut consumption among the New Zealand population has been found to be relatively low (14).

Recently, nuts have received a lot of attention in the lay media regarding the need to 'activate' nuts prior to consuming them. The public have been bombarded with information on various soaking methods to activate nuts where the advocates claim that soaking reduces the anti-nutrient, phytate, allowing greater nutrient bioavailability, decreased digestion discomfort and changes in texture and flavour of the nut (17-21). However, there has been no scientific evidence to support or refute such claims, which may have caused misconceptions among health professionals and the general public. Furthermore, if people believe health benefits are only apparent after nuts are 'activated', the time-consuming process of soaking nuts and the limited availability of pre-soaked nuts and nut products, which tend to be more expensive, could in fact be barriers to regular nut consumption among the New Zealand population.

It is evident from the current research that soaking nuts in the whole form was not effective in reducing phytate concentrations. Conversely, chopping the nuts led to greater reductions in phytate, however mineral content was also compromised. Therefore, there is no evidence to support the claims that activating nuts reduces phytate content to the extent which allows for greater nutrient bioavailability. This finding is

important as it can inform evidence-based guidelines which can now emphasise the lack of evidence that soaking nuts increases mineral bioavailability.

Research in the literature aiming to improve the bioavailability of minerals through soaking has been undertaken in developing countries where phytate concentrations in food are high and mineral contents are relatively low. Therefore, given the higher mineral intakes in the New Zealand context, arguably the phytate:mineral ratio may only be important for certain sectors of the population e.g. vegans and vegetarians, where consumption of phytate containing foods is higher.

In addition, sodium content increased in both whole and chopped nuts when soaked in salt solutions, which contributed a further ~ 4%- 12% of the suggested dietary target (1600 mg/day) for every 30 g of nuts consumed (96). Therefore, individuals who are consuming 30 g or more of soaked nuts daily, are likely to have higher a sodium intake. Given the current sodium intake in the New Zealand population is 3386 mg/ day (well above the recommended upper limit of 2300 mg), the intake of additional sodium from the soaking solution is undesirable (97). Caution should thus be advised for individuals who prefer soaking nuts prior to consumption.

Overall, this study suggests there is no evidence for dietitians to recommend the soaking of nuts prior to consumption in order to improve mineral bioavailability. However, if soaking is preferred, when counselling clients or patients, further exploration would be useful regarding soaking method and minimising use of salt in soaking solution. Ideally, the findings from this study can also be used to clear any misconceptions regarding the necessity of soaking nuts by informing public health messages.

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